THE ASSESSMENT OF RIGHT VENTRICULAR FUNCTION IN INFANTS WITH PULMONARY HYPERTENSION

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ABSTRACT

Pulmonary hypertension is a cause of significant morbidity and mortality in newborn infants. Right ventricular function, or dysfunction, is an important consequence of pulmonary hypertension and may be an important determinant of disease severity.

This work aimed to improve the assessment and management of infants with pulmonary hypertension by:

1. identifying non-invasive measures of right ventricular function in infants
2. determining the mechanisms of right ventricular dysfunction
3. demonstrating the variability of the relationship between RV function and PAP

Five echocardiographic measures were selected to assess RV function; tricuspid valve Doppler inflow, right ventricular output (RVO) , RV myocardial performance index (RV_{MPI}), pulse wave tissue Doppler imaging (PWTDI) and colour tissue Doppler imaging (CTDI). Using a case-control design each measure was performed in a control group of infants with normal cardiovascular function, and a PHT group of infants with elevated pulmonary artery pressure. This design allowed assessment of each measure, and provided normative data for those measures (RV_{MPI}, PWTDI and CWTDI) which had not previously been performed in infants.

All measures were found to be technically feasible, and to provide some quantification of haemodynamic performance. However, the load-dependence of TV Doppler and the global nature of RV_{MPI} and RVO meant that these measures could not be considered pure measures of RV myocardial function alone. By contrast, Tissue Doppler imaging measures allowed separate assessment of systolic and diastolic function. This study was an important first demonstration of the feasibility and application of TDI in an infant disease state. Future studies are indicated to assess the load-dependence of TDI measures in infants, the repeatability of the technique and use of TDI in other infant diseases with myocardial dysfunction.

The mechanisms of RV dysfunction in infants with PHT were investigated by comparison of RV function data between control and PHT groups. Accepting the limitations of the measures used, the results indicated the presence of impaired systolic and early diastolic function in infants with PHT. This finding highlighted the importance of diastolic dysfunction in the failing infant heart, and the usefulness of measures such as TDI which allow assessment of both systolic and diastolic dysfunction. There are also potential therapeutic implications, and the theoretical benefit of drugs with both inotropic and lusitropic actions in this setting was an important area identified for future research.

Finally, no linear relationship was identified between RV function measures and PAP in the PHT group. It was concluded that pulmonary artery pressure should not be used as a proxy measure of RV function in infants and thus emphasised the

importance of directly assessing RV function in infants with pulmonary hypertension.

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DECLARATION

This thesis represents original work carried out by the author, and has not been submitted in any form to any other University. Where materials or practical support have been received, due acknowledgement has been made in the text.

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ABBREVIATIONS

A (velocity)	late diastolic transtricuspid velocity
A' (velocity)	late diastolic myocardial velocity
ACE	angiotensin converting enzyme
AV	atrio-ventricular
BP	blood pressure
CDH	congenital diaphragmatic hernia
CPAP	continuous positive airway pressure
CTDI	colour tissue Doppler imaging
CSA	cross-sectional (valve) area
DMDT	diastolic myocardial displacement time
dP/dT_{max}	maximum change in ventricular pressure with time
E (velocity)	early diastolic transtricuspid velocity
E'	early diastolic myocardial velocity
E_{max}	maximum elastance
ECG	electrocardiogram
EDPVR	end-diastolic pressure-volume relationship
ESPVR	end-systolic pressure-volume relationship
HFJV	high frequency jet ventilation
HFOV	high frequency oscillatory ventilation
HR	heart rate
iNO	inhaled nitric oxide
IVA	(myocardial) isovolumic acceleration
IVCT (ICT)	isovolumic contraction time
IVRT (IRT)	isovolumic relaxation time
IVV	(myocardial) isovolumic contraction velocity
IVS	inter-ventricular septum
K_c	chamber stiffness constant
LA	left atrium
LV	left ventricle
MAS	meconium aspiration syndrome

MPA	main pulmonary artery
MPI	myocardial performance index
MR	magnetic resonance imaging
NNU	neonatal unit
PAP	pulmonary artery pressure
PDA	patent ductus arteriosus
PGE_1	prostaglandin E1
PHT	pulmonary hypertension
PPHN	persistent pulmonary hypertension of the newborn
PVR	pulmonary vascular resistance
P-V	pressure-volume
PWTDI	pulse wave tissue Doppler imaging
RA	right atrium
RAP	right atrial pressure
RCH	Royal Children's Hospital
RV	right ventricle
RV_{MPI}	right ventricle myocardial performance index
RVEF	right ventricular ejection fraction
RVET	right ventricle ejection time
RVFS	right ventricular fractional shortening
RVO	right ventricular output
RVOT	right ventricular outflow tract
S	myocardial systolic ejection velocity
SV	stroke volume
SVC	superior vena cava
TDI	tissue Doppler imaging
TR	tricuspid regurgitation
TV	tricuspid valve

VGAM	vein of Galen malformation
VTI	velocity time integer

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Pulmonary hypertension in the newborn infant

Pulmonary hypertension is defined in this work as elevated pressure in the pulmonary arterial system. Pulmonary hypertension is a common neonatal problem occurring in approximately 1 in 1000 live births [1, 2] and is a cause of significant morbidity and mortality in the neonatal unit [3]. Pulmonary hypertension is commoner in term than preterm infants [4].

Pulmonary vascular resistance, and hence pulmonary artery pressure, is elevated in the fetus and falls rapidly after birth.Pulmonary hypertension present from birth is therefore often referred to as "persistent pulmonary hypertension of the newborn" (PPHN). However, the term PPHN is avoided in the remainder of this thesis as it does not distinguish specific disease processes or pathophysiological mechanisms.

1.1.1 Aetiology of pulmonary hypertension

Pulmonary hypertension in newborn infants may be associated with underlying pulmonary or cardiac disease, or may occur in isolation in which case it is termed idiopathic pulmonary hypertension.

The underlying pathological mechanisms of elevated pulmonary artery pressure may involve one or a combination of the following: failure of normal pulmonary vasodilatation at birth, structural abnormalities of the pulmonary vascular bed, obstructed or reduced pulmonary blood flow and excessive pulmonary blood flow.

These mechanisms of pulmonary hypertension and the disease processes associated with them are listed in Table 1.1, and discussed further below.

Table 1.1: Mechanisms of pulmonary hypertension in the newborn infant and associated disease processes (modified from Ostrea et al [2])

Persistent Pulmonary Vasoconstriction	Perinatal asphyxia
	Meconium aspiration syndrome
	Neonatal respiratory distress syndrome
	Sepsis/pneumonia including group B streptococcal infection
	Bronchopulmonary dysplasia
Abnormal pulmonary vascular bed	Congenital diaphragmatic hernia
	Alveolar-capillary dysplasia
	Pulmonary hypoplasia (e.g. oligohydramnios, thoracic dystrophy)
Obstructed / reduced pulmonary blood flow	Hyperviscosity
	Total anomalous pulmonary venous drainage
	Mitral valve stenosis
	Hypoplastic left heart
	Obstructed left ventricular outflow (e.g. aortic coarctation, aortic valve stenosis)
Excessive pulmonary blood flow	Systemic-to-pulmonary shunts (e.g. ventriculo-septal defect, patent ductus arteriosus)
	Systemic arterio-venous malformations (e.g. vein of Galen malformation)

1.1.1.1 Persistent pulmonary vasoconstriction

Before birth the pulmonary vasculature is constricted, PVR is high and only 8% of total cardiac output passes through the pulmonary circulation. Under normal circumstances PVR falls rapidly at birth as a consequence of lung inflation and increasing oxygen levels. Failure of these transitional mechanisms at birth, leading to persistent pulmonary vasoconstriction, may be due to a combination of three inter-related factors; hypoxia, impaired lung inflation and inflammation [5].

Hypoxia reduces production of the vasodilators nitric oxide and prostacyclin, reduces smooth muscle sensitivity to nitric oxide and increases production of the vasoconstrictor endothelin-1 [6]. Hypoxia may occur due to a global perinatal hypoxic insult (perinatal asphyxia), or impaired gas exchange (as in meconium aspiration, pneumonia, or respiratory distress syndrome) or more rarely cyanotic heart disease.

Impaired lung inflation prevents the normal stretching and unkinking of pulmonary blood vessels and shear-stress-triggered release of vasodilating cytokines NO and prostacyclin [7, 8]. Impaired lung inflation may contribute to PHT in meconium aspiration and respiratory distress syndrome [7-9].

Inflammation associated with persistent pulmonary vasoconstriction at birth is seen in conditions such as sepsis, pneumonia (especially group B streptococcal infection) and meconium aspiration [7]. Inflammatory cytokines produced in the lungs in these conditions may act directly as pulmonary vasoconstrictors [6].

1.1.1.2 Abnormal pulmonary vascular bed

Infants may be born with abnormal pulmonary vascular architecture leading to a fixed elevation of PVR at birth. The abnormality may be limited to the pulmonary blood vessels alone, as in the rare condition alveolar capillary dysplasia [10], or associated with pulmonary hypoplasia as in oligohydramnios, thoracic dystrophy and congenital diaphragmatic hernia (CDH) [4, 11]. In the case of CDH pulmonary arteries have reduced cross-sectional areas, increased medial wall thickness and abnormal muscularisation, all of which contribute to elevated PVR [12, 13].

1.1.1.3 Obstructed pulmonary blood flow

Obstructed pulmonary blood flow represents a relatively rare mechanism of pulmonary hypertension in which the pulmonary arterial vessels may be of normal structure and function, but as a consequence of distal obstruction to flow, high pressure is generated proximally. This occurs in pulmonary venous obstruction (e.g. total anomalous pulmonary venous drainage) and structural and functional obstruction of the left heart (e.g. hypoplastic left heart, critical aortic stenosis, aortic coarctation) [14, 15].

1.1.1.4 Excessive pulmonary blood flow

Abnormally high flow through normal pulmonary vasculature may also be a cause of pulmonary hypertension. High flow states may result from systemic-to-pulmonary shunts as in PDA and ventricular-septal defect, or rarer arterio-venous fistulae such as vein of Galen aneurysmal malformation (VGAM) [16].

It is important to note that in an individual disease state the pathophysiology of pulmonary hypertension may be multi-factorial. In congenital diaphragmatic hernia, for example, elevated PVR results both from abnormal vessel architecture and from hypoxia-induced pulmonary vasoconstriction [11, 17]. Furthermore, the underlying mechanisms of pulmonary hypertension in a newborn infant are dynamic. Initial pulmonary vasoconstriction, due to failure of smooth muscle relaxation, may be followed by abnormal remodelling of the vessel architecture. Specifically, prolonged hypoxia causes smooth muscle cells to change phenotype, altering the relative levels of contractile proteins and switching myocytes from contractile elements to proliferative cells. Microscopically, vessels demonstrate thickening of the vascular media, hyperplasia and hypertrophy of smooth muscle and excess extracellular matrix deposition. The result is increased vessel stiffness, reduced distensibility and an irreversible increase in PVR [18, 19].

1.2 Cardiopulmonary consequences of pulmonary hypertension

Increased pulmonary artery pressure has two major consequences for circulatory function in the newborn: hypoxaemic right-to-left shunting and myocardial dysfunction.

1.2.1 Right to left shunting

In infants with severe pulmonary hypertension, elevations in pulmonary arterial pressure above systemic arterial pressure create a pressure gradient for blood flow

across the patent ductus, from the main pulmonary artery to the proximal descending aorta. The degree of ductal shunt will be directly related to pulmonary arterial and systemic arterial pressure differences.

Low pulmonary flow, due to high pulmonary artery pressure, also leads to reduced pulmonary venous return and low left atrial pressures. Meanwhile, right atrial pressure is elevated due to increased afterload on the right heart. This pressure gradient between atria causes right to left shunting through the patent foramen ovale and mixing of venous and arterial blood in the left atria.

Right-to-left ductal and inter-atrial shunting in PHT mimics the fetal circulation and led Gersony to use the term "persistent fetal circulation" in his first descriptions of infants with pulmonary hypertension [20]. The clinical consequence of these right-to-left intra and extra-cardiac shunts is systemic hypoxaemia and the classical presentation of a cyanosed infant without significant respiratory distress, as first described by Chu et al [21].

1.2.2 Myocardial dysfunction

The second major feature of the circulation in pulmonary hypertension is myocardial dysfunction. This was first reported by Rowe and Hoffman in 1972, in three infants who presented with the classical features of pulmonary hypertension at birth; cyanosis, tachypnoea and heart failure within the first 24 hours of life. These infants had clinical evidence of congestive heart failure and poor ventricular function demonstrated by ventricular angiography, together with ischaemic changes on

electrocardiograms [22]. Reimenschneider et al in 1979 confirmed the finding of myocardial dysfunction in newborn infants with pulmonary hypertension, reporting a series of infants in whom cyanosis was accompanied by clinical evidence of congestive heart failure and hypotension [23]. The concept of myocardial dysfunction in infants with pulmonary hypertension is central to the studies in this thesis, and is therefore expanded below.

1.3 Mechanisms of myocardial dysfunction in pulmonary hypertension

To understand the mechanisms of myocardial dysfunction in pulmonary hypertension it is useful to first consider the normal physiology and anatomy of the right ventricle.

1.3.1 Normal physiology and anatomy

RV function, at its most basic, may be divided into systolic and diastolic function. Systolic function refers to ventricular contraction and ejection, whilst diastolic function refers to ventricular relaxation and filling [24].

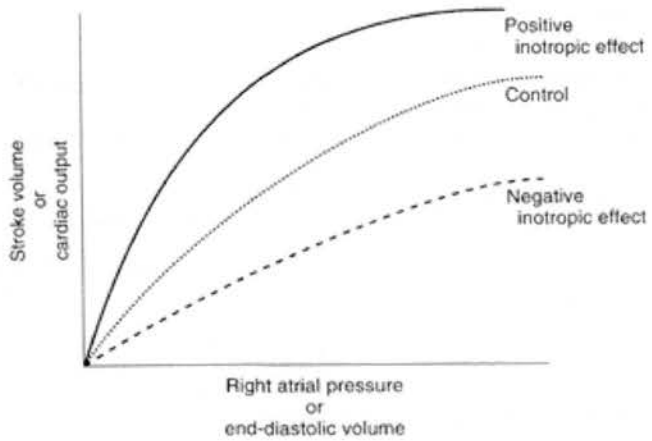
1.3.1.1 Systolic function

Systole consists of an initial isovolumic contraction phase, during which myocardial contraction increases RV pressure without volume change, and is followed by a more prolonged ejection phase during which ventricular volume reduces and blood is ejected across the pulmonary valve. Systolic function is determined by the inherent

contractile function of the myocardium, termed "contractility" or "inotropy", as well as the loading conditions of the heart i.e. preload and afterload. The Frank-Starling mechanism describes how as loading increases (leading to increased end-diastolic pressure and volume) so the force of contraction (contractility), and resultant stroke volume, will increase too. This gives rise to the relationship demonstrated in Figure 1.1.

Changes in inherent contractility will alter this relationship independently by shifting the relationship up or down. At a cellular level both length-dependent and inotropy-dependent mechanisms both alter contractile function by changing calcium handling within the myocyte [25]. Techniques used in research and clinical practice often cannot distinguish the relative contributions of loading and inotropy to systolic function, and therefore in this thesis the term "contractility" is used to refer to combined effects of these on contractile function. It is important to make the distinction, however, that loading conditions may also **directly** affect the measures of systolic function (independent of an effect on contractility) and that this must be taken into account when interpreting such measures, as is discussed later.

Figure 1.1: The Frank-Starling relationship; effects of loading and inotropic state on ventricular function



1.3.1.2 Diastolic function

Diastole is dependent on both myocardial relaxation and chamber stiffness, or compliance, as well as atrial contraction, tricuspid valve function and heart rate. Myocardial relaxation is an active process which involves myocardial inactivation i.e. a reduction in contractile force due to uptake of calcium into the sarcoplasmic reticulum of the myocytes [26]. Chamber stiffness is determined by the passive properties of the ventricular wall dependent on the cellular cytoskeleton, extracellular matrix, wall thickness and chamber geometry [27].

Diastole consists of three distinct phases. 1) *Isovolumic relaxation* occurs first and is characterised by a fall in ventricular pressure without a volume change, after closure of the pulmonary valve and before tricuspid valve opening. The term *isovolumic relaxation* is rather misleading as this phase involves changes in ventricular

geometry which require synchronous contraction and relaxation of different ventricular regions[26]. 2) Next is the early (E) filling phase, immediately after tricuspid valve opening, which is dependent on the active myocardial relaxation and passive properties of the ventricle as discussed above. 3) Finally in late diastole atrial contraction occurs producing a final (A) phase of filling before systole.

1.3.2 Pathophysiology of myocardial failure in pulmonary hypertension

Having briefly considered normal myocardial function, the mechanisms of myocardial dysfunction in pulmonary hypertension are now discussed. For the purpose of this work the term "myocardial dysfunction" refers to systolic and/or diastolic dysfunction.

Changes in myocardial function in response to acute and chronic pulmonary hypertension have been studied in a variety of models; principally adults and animals. In acute pulmonary hypertension the first response of the RV to increased afterload (pulmonary artery pressure) is an increase in systolic contractility. This has been demonstrated using invasive catheter techniques to measure elastance, a "gold-standard" measure of contractility, in newborn lambs with respiratory distress syndrome and associated pulmonary hypertension, and in adult animal models of acute pulmonary hypertension. [26-28].

However, acute increases in afterload which cause excessive dilatation of the RV will increase its volume beyond the maximal point on the Frank-Starling relationship and lead to reductions in stroke work. Left ventricular function is also affected under these circumstances by the process of inter-ventricular dependence. As the RV dilates the interventricular septum is displaced, and the LV is compressed, impairing LV filling and contraction. The result may be acute and fatal biventricular failure as has been demonstrated in a canine model of pulmonary embolus where acute, severe increase in afterload leads to cardiogenic shock. [28].

Chronic exposure to increased afterload leads to right ventricular hypertrophy, which has been demonstrated to occur within 96 hours in animal models [29]. The hypertrophied RV becomes more concentric, thick-walled and less compliant. These changes are partially adaptive and compensatory, and may initially improve function. However, progressive hypertrophy may be a maladaptive process and detrimental to cardiac function [30]. In rat models of pulmonary hypertension Buermans et al have demonstrated that increasing pulmonary artery pressure tips the balance of RV function from compensated hypertrophy to progressive cardiac failure [31]. This "pivotal divergence" from a compensated ventricle with preserved function to a failing ventricle appears to involve the activation of pro-apoptotic gene pathways, together with changes in myocardial energy metabolism and calcium signaling contributing to impaired ventricular function [32]. Animal models have identified that it is RV *diastolic* function which is particularly affected in the failing ventricle, demonstrated by increased RV stiffness and prolonged isovolumic relaxation times [33, 34]. Hypertrophy and remodelling of the RV also impairs LV function as it

continues to displace the interventricular septum. In addition, the enlarged RV has increased metabolic demands which may not be met in a situation of ongoing hypoxia, with resultant risk of myocardial ischaemia and worsening failure [33].

In summary, the term "myocardial failure" when applied to the setting of pulmonary hypertension in animal models at least, refers to a combination of systolic and diastolic dysfunction, secondary left ventricular dysfunction and myocardial ischaemia.

Do the same mechanisms of cardiac failure occur in newborn infants with pulmonary hypertension? Despite 35 years passing since the original reports of myocardial dysfunction in infants with pulmonary hypertension, little is known about the mechanisms of RV failure in infants other than can be extrapolated from animal and adult data. No studies have yet investigated whether RV failure in newborn infants, or an age-appropriate animal model, involves both systolic and/or diastolic dysfunction. These are potentially important areas to investigate as they may help clinicians choose better, more targeted therapies.

1.3.3 The spectrum of myocardial dysfunction and contributing factors

Not every ventricle demonstrates the same response to elevated pulmonary artery pressure. The variable ability of the right ventricle to function, or fail, in the face of high afterload was first recognised in the earliest report of myocardial failure in PPHN by Riemenschneider et al. Thirteen infants were reported who had severe

central cyanosis, but only ten of these had congestive heart failure and hypotension. It was reasoned that there existed a "spectrum" of myocardial dysfunction in the face of increased afterload [23]. This also highlights the fact that disease severity in pulmonary hypertension is determined by the degree of myocardial function or dysfunction, and not the absolute pulmonary pressure. Infants with the same absolute level of pulmonary hypertension may be profoundly unwell or entirely asymptomatic [34, 35]. This peculiarity is well recognised by clinicians experienced in treating infants with pulmonary hypertension, yet has been little reported in the literature.

Variability in RV function has two major implications for the assessment and management of infants with pulmonary hypertension:

1. Measurement of pulmonary artery pressure alone does not allow prediction of the degree of RV function or dysfunction in pulmonary artery pressure

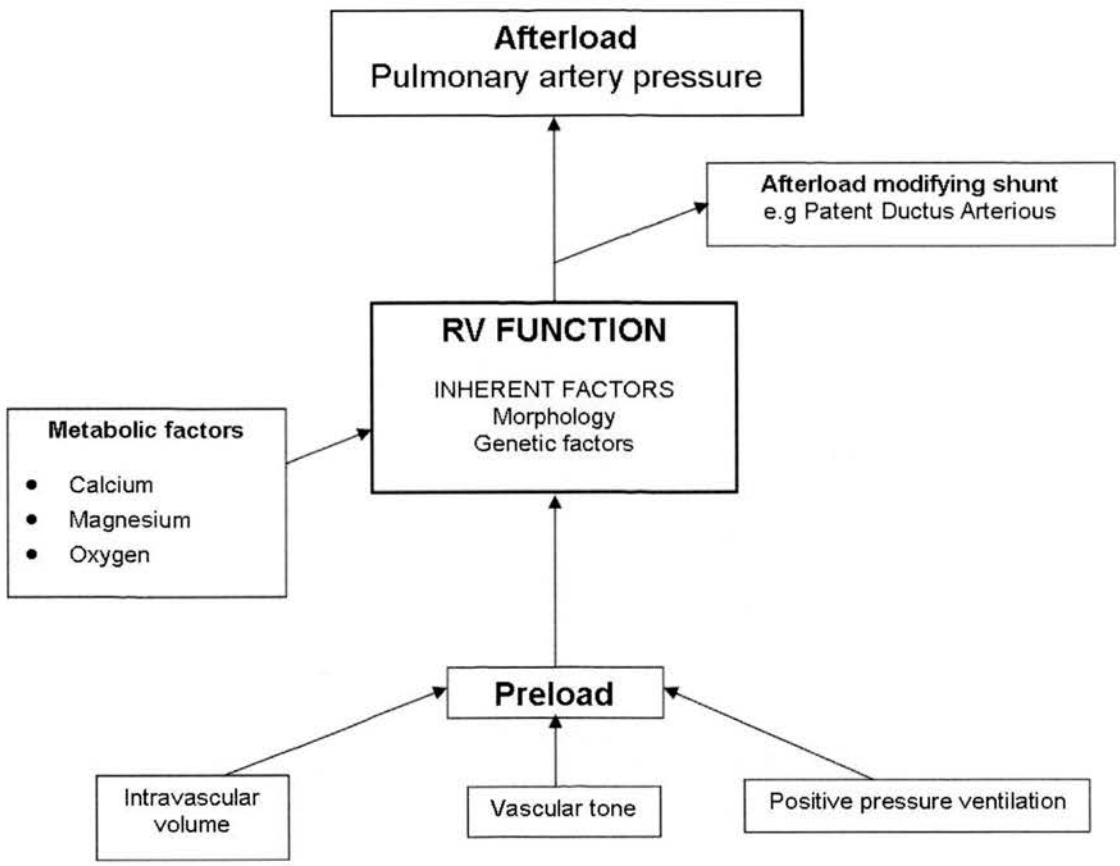
2. Direct measurement or RV function in pulmonary hypertension provides an assessment of severity of illness

The variable response of the RV to increased afterload is a central concept in this thesis and underlies the importance of direct assessment of RV function in pulmonary hypertension.

What are the reasons for the variability of RV function in the face of increased PVR?

RV function in a sick infant may be determined by many variable factors. Increased afterload, i.e. raised pulmonary artery pressure, will undoubtedly be an important determinant of function, other factors however are also important in determining RV function, as shown in Figure 1.2 and discussed below. Specifically, these include mechanical factors which may influence afterload and preload, metabolic factors and morphology and molecular characteristics of the heart itself.

Figure 1.2: Factors contributing to variable RV function



1.3.3.1 Preload and ventricular function

Preload, or venous return to the heart, is one of the most important determinants of ventricular function, via the Frank-Starling Mechanism. Alterations in preload may produce variable RV function despite a constant afterload. Preload is itself dependent on intravascular volume and on venous and arterial vascular tone. Shock, hypovolaemia, sepsis and use of vasoactive drugs may all alter preload and hence ventricular function.

1.3.3.2 Positive pressure ventilation and ventricular function

Sick infants with severe pulmonary hypertension frequently require respiratory support in the form of conventional or high frequency ventilation. Use of positive pressure ventilation may produce variable effects on ventricular function by altering preload, afterload and ventricular function directly. Adult and animal work suggests that increasing mean airway pressure will tend to reduce preload, increase afterload and directly impair ventricular function [36, 37]. Studies in infants are limited but those performed have shown increased pulmonary vascular resistance, reduced myocardial contractility (systolic function) and an overall reduction in cardiac output as airway pressures are increased within the clinical range [36, 37]. There are however no studies specifically investigating the effects of ventilation in infants with pulmonary hypertension in whom abnormal pulmonary vasculature may be differently susceptible to changes in airway pressure.

1.3.3.3 Afterload modification: right-to-left shunts

Another important factor which may determine RV performance in infants with pulmonary hypertension is the presence or absence of a right-to-left shunt which may reduce the total RV afterload. This may take the form of a patent ductus arteriosus, or a ventricular or atrial septal defect. These allow blood to shunt from the RV to the systemic circulation when pulmonary pressures rise above systemic pressures, decompressing the RV to prevent further dilatation and therefore maintaining function on the ascending limb of the Frank-Starling curve. Indeed, in infants with pulmonary hypertension secondary to congenital diaphragmatic hernia, use of prostaglandin E1 has been advocated to maintain ductal patency as a means of maintaining good RV function. This measure might contribute to improved survival in these infants [17, 38].

1.3.3.4 Inherent factors: ventricular morphology and genetics

Inherent characteristics of the right ventricle itself may influence function. It has already been discussed that ventricular morphology changes in response to persistent increases in afterload, and that this may alter function. Animal models have demonstrated that the RV becomes hypertrophied and more concentric in shape in response to increased PVR. This is thought to be an adaptive response to create a chamber, more like the LV, which is less compliant and better able to generate higher end-systolic pressures and preserve RV function to bring about improved survival [39]. However, as previously discussed, cardiac hypertrophy in pulmonary hypertension can progress from an adaptive compensatory process to a situation of worsening cardiac failure [31]. In this way progressive remodelling may partially

explain observed variation in right ventricular function, with initial improvement followed by worsening failure.

Genetic and molecular factors may also contribute to variable RV function in response to increased afterload. Abraham et al have suggested a role for polymorphisms of the angiotenisn converting enzyme (ACE) gene in producing variable RV response in adult hypertension [40]. Thirty-two patients with primary pulmonary hypertension were studied and those with ACE DD genotype ([D]eletion versus [I]nsertion of a polymorphism of part of the gene) demonstrated preservation of cardiac output compared to non-DD patients. Differential ACE genotype expression may therefore play a role in variable RV function in pulmonary hypertension. However, as yet, neither ACE genotype, nor other candidate genes have been studied in infant pulmonary hypertension.

1.3.3.5 Metabolic factors

In sick infants with severe pulmonary hypertension co-existent metabolic derangements may be responsible for changes in myocardial function. Hypocalcaemia, hypomagnesaemia, hypoxia and acidosis may all directly influence myocardial function and contribute to variation in function, independent of changes in afterload [41, 42].

In summary, variable RV function in pulmonary hypertension may be a consequence of many varied factors which may influence ventricular function, not simply afterload alone. This emphasises the point made at the beginning of this section; that

RV function in pulmonary hypertension cannot be predicted solely by measurement of pulmonary artery pressure. Instead, assessment of the clinical severity of the pulmonary hypertension requires direct, repeated assessment of right ventricular function.

1.4 Assessment of RV function in the newborn infant

RV function assessment in infants with pulmonary hypertension is important for the following reasons:

- **RV dysfunction is a key consequence of pulmonary hypertension and a determinant of disease severity**

- **RV function is variable and dependent on many factors of which PVR is just one. Measurement of pulmonary artery pressures alone may therefore be an inadequate predictor of RV dysfunction or disease severity**

- **To identify when therapies are required and assess the response to therapy**

Accepting the importance of assessing RV function in infants with pulmonary hypertension the remainder of this chapter will discuss potential means of assessing

myocardial function in infants, and their prior use in the setting of pulmonary hypertension.

An ideal measure of RV function in infants would be:

- *Quantifiable*
- *Accurate and repeatable*
- *Non-invasive and safe*
- *Rapidly performed at the cotside*
- *Able to quantify the different components of myocardial function, i.e.:*
    - *In systole allow measurement of contractility independent of loading conditions*
    - *In diastole allow separate measurement of myocardial relaxation (active) and compliance (passive), independent of loading conditions*

Unfortunately no ideal measure of RV function exists. With the above criteria in mind this section discusses the following means of RV assessment:

- Indirect clinical assessment of cardiac function
- Invasive conductance catheters
- Cardiac magnetic resonance imaging
- Echocardiographic assessment

The deficiencies of methods in current clinical practice will be highlighted and alternative techniques will be considered, including new echocardiographic techniques whose use in the clinical setting forms the basis of this thesis.

1.4.1 Indirect clinical assessment of cardiac function

Infants with severe pulmonary hypertension are usually managed in neonatal or paediatric intensive care units. Routine monitoring in these settings includes heart rate, blood pressure, oxygen saturation, respiratory rate and clinical assessment of perfusion including capillary refill time. Together with laboratory adjuncts (paO_2, pH, serum lactate) these measures provide a broad indication of general condition but are poor measures of cardiac function [43].

Measurement of pre- and post-ductal oxygen saturations can give some indication of pulmonary artery pressure relative to systemic pressure. A widening pre-post ductal saturation difference with lower post-ductal saturations indicates supra-systemic pulmonary artery pressures and increasing right to left shunt. However, this provides no absolute quantification of PA pressure and can give no indication of resultant RV myocardial function.

These measures, though arguably the easiest and most widely used indicators of circulatory function in infants with PHT are too non-specific to be used as the sole indicators of RV function.

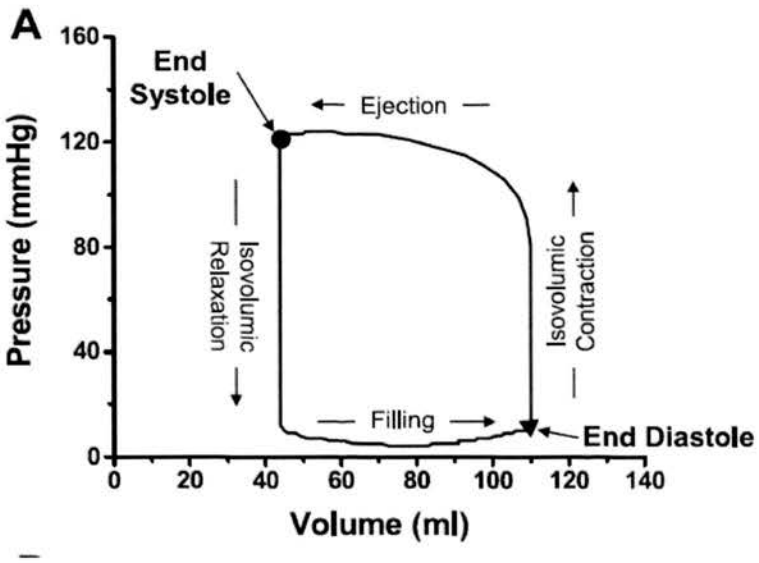
1.4.2 Invasive conductance catheter measures of RV function

Invasive conductance catheters inserted via central veins into the ventricle are the gold standard measure of ventricular function.

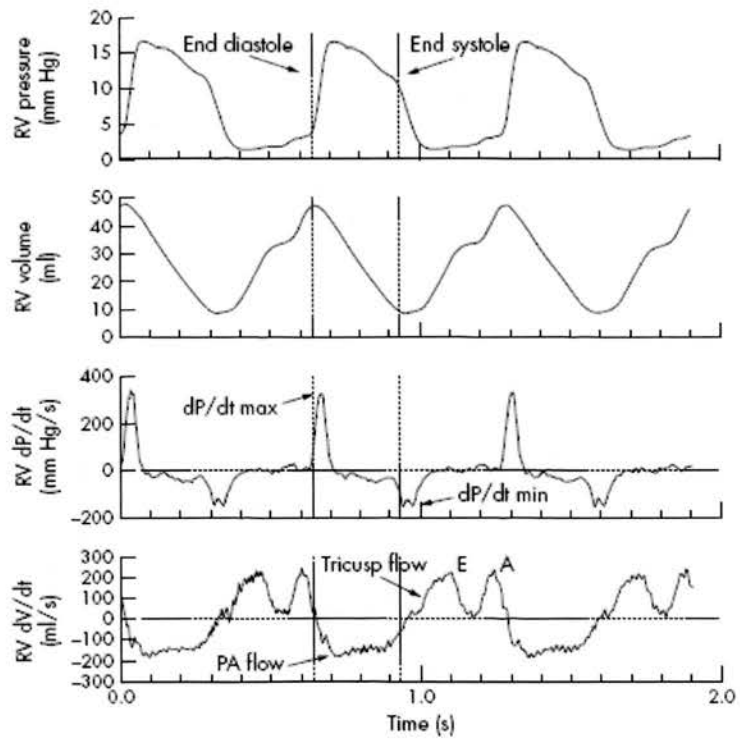
Using catheters with high fidelity micro-anemometer tips, the relationships of pressure and time, and pressure and volume can be obtained during each cardiac cycle as shown, for example from the left ventricle, in Figure 1.3.

Figure 1.3: Pressure-volume and pressure-time relationships obtained using invasive conductance catheters inserted into the ventricle

1.3 A: Example of the ventricular pressure-volume relationship. From Burkhoff et al[44]



1.3 B: Ventricular pressure-time relationship in a normal sheep. From Bleeker et al [45]



Systolic function (i.e. myocardial contractility) is assessed using two principal indices; the maximum rate of change of pressure with time in the isovolumic phase of systole (dP/dT_{max}) and maximum elastance (E_{max}) [44].

dP/dT_{max} is obtained from the pressure-time relationship (as seen in Figure 1.3), and is a highly sensitive marker of changes in contractile state[46]. However, dP/dT_{max} also demonstrates a degree of load dependence which limits its interpretation as a pure measure of contractile function [47].

E_{max} is obtained from analysis of the end systolic pressure-volume relationship (ESPVR) at the top left hand corner of the P-V loop. A series of P-V loops at different loading conditions can be created by reducing preload, for example by balloon occlusion of the inferior vena cava. E_{max} is the slope of the steepest tangent to the loop at this point (see Figure 1.4), and in the RV represents the best load-independent measure of contractile function [48]. However, E_{max} appears to be less sensitive to inotropic manipulations in contractile state than dp/dt max [49]. Despite their limitations dP/dT_{max} and E_{max} represent the most sensitive and load independent measures of contractile function available for in-vitro.

The components of diastolic function are *myocardial relaxation* and passive properties (*chamber stiffness*) both of which may also be assessed by catheter measures. There are two principal measures of *myocardial relaxation*; 1) dP/dt_{min} is an instantaneous value reflecting the maximum rate of pressure fall, and 2) τ is the

time constant of isovolumic relaxation. Additionally, the isovolumic relaxation time (IVRT) is the time interval between pulmonary valve closure and tricuspid valve opening may be measured and is a partially load-dependent measure of diastolic function [24],[27]. *Chamber stiffness* is assessed by analysis of the end-diastolic pressure-volume relationship (EDPVR), i.e. the bottom right corner of the pressure-volume loop, as shown in Figure 1.4. The slope of a tangent drawn to the curve at EDPVR is the *chamber stiffness constant* (K_c). Of note chamber stiffness is also dependent on the volume of the ventricle and as such K_c is dependent on inherent properties of the ventricle and ventricular volume [7].

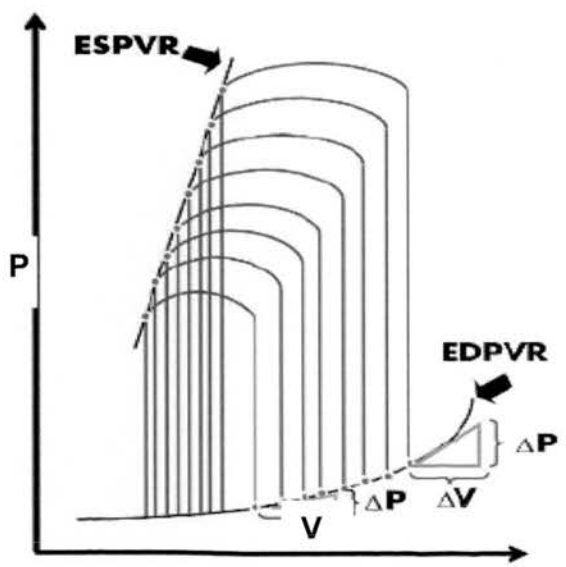
Figure 1.4: Evaluation of systolic and diastolic function from pressure-volume loops

At end systolic pressure-volume relationship (ESPVR) the slope of the steepest tangent to the pressure-volume curve represents maximal elastance (E_{max})

.

At the end diastolic pressure-volume relationship (EDPVR) the slope of the tangent ($\Delta P/\Delta V$) is the stiffness constant (K_c) a measure of chamber stiffness.

Diagram from Leite-Moreira [24].



Although these gold standard measures provide the most accurate means of in vitro assessment of myocardial function they are unsuitable for use in the clinical setting

especially in the sick infant. Insertion of intra-cardiac catheters in small infants is technically challenging and is associated with significant and potentially fatal risks. Specifically, invasive catheters are associated with myocardial or vessel perforation, infection, thrombosis, pericardial effusion, arrhythmia, and pulmonary hypertensive crisis [50-52]. For these reasons use of catheters to assess RV function in infants with pulmonary hypertension cannot be considered for research purposes in this thesis or recommended as a routine clinical tool.

1.4.3 Cardiac Magnetic Resonance Imaging

In the past decade there has been great interest in the use of magnetic resonance imaging (MRI) to assess cardiac structure and function. The development of modern scanners with high temporal and spatial resolution has enabled studies of newborn infants [53]. This may be particularly useful for defining cardiac anatomy in congenital heart disease. Cardiac MRI also allows accurate assessment of ventricular volume and blood flows throughout the cardiac cycle using ECG-triggered phase velocity mapping [45]. However, MRI has significant practical drawbacks which prevent its clinical use as a repeatable assessment of RV function in infants with pulmonary hypertension: 1) few suitable scanners and experienced personnel exist, 2) many infants with severe pulmonary hypertension are too unstable to consider moving them to an MRI scanner for a lengthy scan [12], 3) it is impracticable to transfer infants repeatedly for multiple assessments as may be required during the course of the illness.

1.4.4 Echocardiographic assessment of ventricular function

Echocardiography (cardiac ultrasound) meets many of the criteria for a useful measure of RV function as discussed at the beginning of this chapter. Specifically the technology is safe, well tolerated, non-invasive and readily available in many neonatal units [54].

A number of different echocardiographic measures can be made. This thesis is based on assessment of RV function using some of these measures. In this section each potential measure is discussed with regard to its current use, suitability as a measure of myocardial function and practicality. The measures discussed below are:

- Qualitative assessment of ventricular function using 2-d echocardiography
- Volumetric assessment: Ejection fraction and fractional shortening
- Doppler calculation of right ventricular output
- Doppler assessment of pulmonary artery pressure
- Doppler assessment of tricuspid valve flow
- Doppler derived myocardial performance index
- Tissue Doppler imaging of myocardial function

1.4.4.1 Qualitative assessment of ventricular function from 2-dimensional echocardiography

This is the most commonly used technique employed in the clinical setting to assess infant RV function. Subjective, qualitative assessment of ventricular function is made from 2-dimensional images of the heart obtained from apical and parasternal (long and short axis) views. Function, in different regions of the ventricular free

walls and septum, is classified as normal or mildly, moderately or severely impaired, or else hyperdynamic. The technique is rapid and easily performed with even the most basic echocardiography equipment. However the technique is subjective with high inter-user variability [55], and makes no distinction between systolic or diastolic function and no accurate quantification of function. Though in widespread use, this technique is a poorly reproducible measure of function and certainly cannot be considered a "gold-standard" measure.

1.4.4.2 Volumetric assessment: Ejection fraction and Fractional shortening

These are measures of systolic function based on changes in ventricular dimensions during the cardiac cycle and are widely used in clinical practice to assess LV function. Attempts have been made to measure RV ejection fraction (RVEF) in newborn infants using an area-length method involving measurements from apical views of the ventricle [56]. However, these simplified attempts to estimate RV volume are inaccurate due to the complex, asymmetric geometry of the RV. They are also time consuming and observer dependent [57]. In addition these measures do not distinguish systolic and diastolic function and therefore provide little insight into the mechanisms of ventricular dysfunction. Indeed, it is well recognised that in 30-50% of adults with heart failure, left ventricular failure ejection fraction remains normal despite the presence of significant diastolic dysfunction [58].

1.4.4.3 Doppler assessment of right ventricular output

Pulse wave Doppler can be used to assess the timing and velocity of blood flow within the heart. These measurements can be used to calculate ventricular outputs and provide some indication of myocardial function. Flow across the pulmonary valve (right ventricular output, RVO) or aortic valve (left ventricular output, LVO) are calculated by multiplication of Doppler velocity time intervals with heart rate and the cross sectional area of the valve (measured using two dimensional echocardiography). Calculation of ventricular outputs, using echocardiography in normal infants, demonstrates reasonable agreement with simultaneous invasive measures [59, 60].

Two existing studies have measured RVO in infants with pulmonary hypertension: Walther et al have demonstrated that right ventricular output was reduced in preterm infants with pulmonary hypertension in the setting of respiratory distress syndrome [8]. Evans and Kluckow replicated this finding in preterm respiratory distress syndrome but found no specific association between RVO and pulmonary artery pressure when multivariate analysis was performed [61]. There are no prior studies of echocardiographically-assessed RVO in term infants with other-cause pulmonary hypertension.

The presence of atrial or ductal shunts complicates interpretation of RVO as a measure of cardiac performance and systemic blood flow. This has led Kluckow et al to propose the use of SVC flow, also measured echocardiographically, as a shunt-independent measure of systemic blood flow [62]. SVC flow represents a relatively

fixed proportion of total systemic blood flow, irrespective of the presence of atrial or ductal shunting. SVC flow has been demonstrated to be low in a proportion of preterm infants and to be associated with IVH in this group [63, 64]. However, no studies have assessed SVC flow specifically in infants with PHT. One reason for this may be the technical difficulty of imaging the SVC and obtaining Doppler measures of SVC flow beyond the first 24 hours of age.

A major drawback of any measure of cardiac output, be it RVO or SVC flow, is that these are global measures of cardiovascular function which are dependent on preload, afterload, and myocardial function (both systolic and diastolic) and therefore provide limited information about myocardial function alone. In addition, measurement of RVO is cumbersome involving many steps at which error may be introduced and multiplied (i.e. measurement of valve diameter and calculation of velocity-time integrals). This makes the technique prone to high user variability [65].

1.4.4.4 Doppler assessment of Tricuspid inflow

Measurement of inflow velocities across the atrio-ventricular valves allows some assessment of diastolic function. Doppler flows across the atrio-ventricular valves consist of an E wave in early diastole and a later A wave. The E wave represents ventricular filling immediately after opening of the AV valve and its velocity is dependent on atrial pressure (preload), active myocardial relaxation and the compliance of the ventricle. The later A wave is due to active atrial contraction prior to closure of the tricuspid valve.

Tricuspid inflows have been used to assess RV function in adults with pulmonary hypertension, in whom a pattern of reduced E wave velocity and reduced E:A ratio, has been interpreted as demonstrating increased myocardial stiffness (reduced compliance) and impaired relaxation [66].

Although atrio-ventricular valve Doppler is established in clinical use to assess diastolic function in adult and paediatric disease [67], there are to date, no studies specifically reporting the use of tricuspid valve Doppler to assess RV function in infants with pulmonary hypertension.

In addition there are limitations to use of this technique in newborn infants. First tricuspid inflow velocities are highly preload dependent and cannot therefore be interpreted as demonstrating myocardial function or compliance alone [26]. Furthermore, in infants there is a physiological change in velocities after birth which complicates interpretation. In later infancy, childhood and adulthood E waves dominate (E:A ratio >1) indicating that the majority of ventricular filling occurs early in diastole, however in the newborn E wave velocities are lower than A wave velocities [68] (so-called E-A reversal), reflecting lower RV compliance, and dependence on atrial contraction to ensure ventricular filling. A final important factor limiting use of tricuspid valve Dopplers in infants is fusion of the E and A wave at higher heart rates which can make measurement of individual velocities difficult.

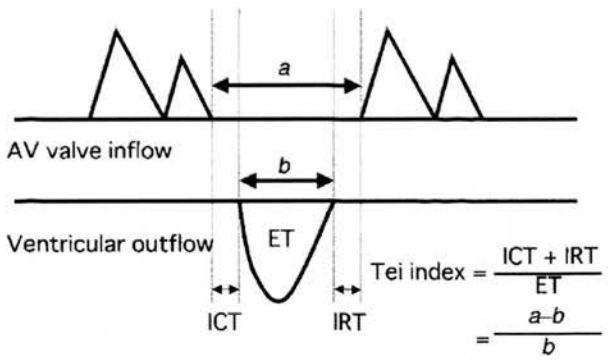
1.4.4.5 Doppler-derived myocardial performance index

The myocardial performance index (MPI), or eponymously named Tei index, is calculated from Doppler flows across the inlet and outlet valves (tricuspid and pulmonary in the case of the RV) and has been proposed as a "global" measure of both systolic and diastolic function [69-71]. The MPI is calculated from the equation:

$$\frac{\text{ISOVOLUMIC RELAXATION TIME + ISOVOLUMIC CONTRACTION TIME}}{\text{RV EJECTION TIME}}$$

In practice the index is calculated using the RV ejection time and the time interval from the cessation to onset of diastolic filling, as shown in Figure 1.5 below.

Figure 1.5: Calculation of the Myocardial Performance Index (Tei Index)



a, period from cessation to beginning of tricuspid inflow; b, period of RV ejection. ET, RV ejection time; ICT, isovolumic contraction time; IRT, isovolumic relaxation time.

MPI was proposed as a rapid means of quantifying ventricular function which can be easily obtained from quick measurements and limited echocardiographic views. One of the major advantages of the index is that it is time interval based and therefore not dependent on angle of insonation of the Doppler beam. In addition the index demonstrates good inter-observer variability and heart rate independence including in normal infants and in the fetus [72].

MPI has been used in RV assessment in adults and children with pulmonary hypertension and in a newborn piglet model of hypoxia-induced pulmonary hypertension [73-75]. In all of these PHT states RV_{MPI} is consistently elevated and has therefore been suggested to provide a practical quantitative measure of global ventricular function. No studies have yet assessed MPI as a measure of ventricular function in newborn infants with pulmonary hypertension.

There are, however, limitations to MPI as a measure of ventricular function. Although proposed as a "global" measure, this in itself is a drawback: an elevated MPI does not indicate whether systolic, diastolic function, or indeed both are impaired. Furthermore the MPI appears to be significantly load dependent i.e. changes in MPI may indicate simply a change in preload or afterload and not ventricular function [76]. Despite its ease of measurement and the attractiveness of a quantifiable measure of ventricular function, the MPI must therefore be interpreted cautiously.

1.4.4.6 Tissue Doppler imaging of myocardial function

Tissue Doppler imaging (TDI) is a new form of imaging which potentially provides the most useful direct echocardiographic measure of ventricular function to date. Use of this technique in infants is a major focus of the work presented in this thesis. TDI is therefore discussed in detail below.

Traditionally, Doppler technology has been used to assess *blood flows* within the heart and blood vessels and is based on reflection of ultrasound from moving erythrocytes. TDI technology, however, assesses not the velocity of blood flows but the velocity of the *myocardium*. TDI myocardial velocities are considered to provide quantitative measures of ventricular function. The ability to perform TDI has resulted from the development of echocardiographic machines capable of filtering out low amplitude, high frequency Doppler signals reflected from erythrocytes, and selecting out the low frequency signals form the myocardium [77].

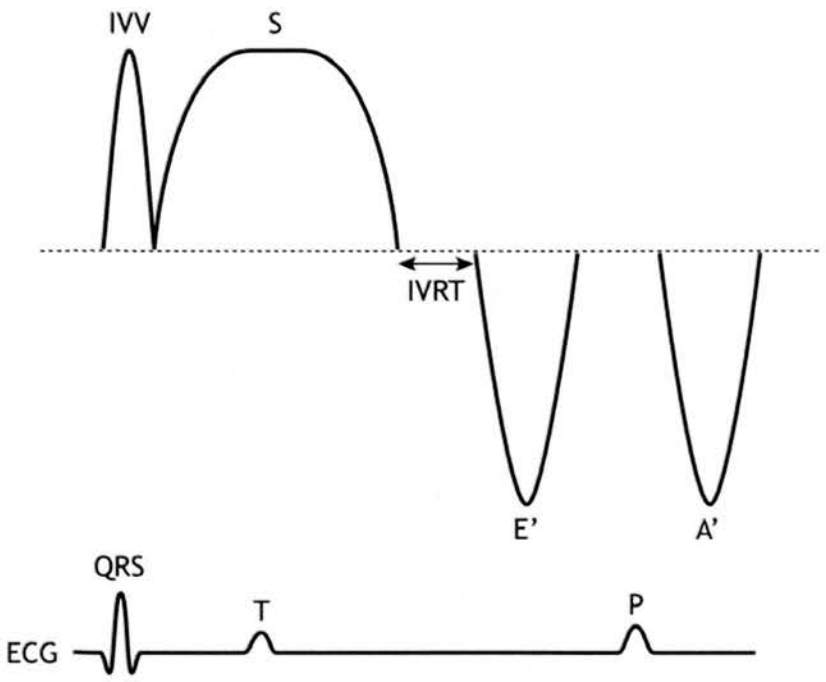
Two forms of tissue Doppler imaging exist: pulse wave TDI (PWTDI) and colour TDI (CTDI). Pulse wave tissue Doppler is simpler and involves use of pulsed wave Doppler to measure the velocity of a segment of myocardium in real-time. A Doppler profile of velocities throughout each cardiac cycle is obtained from which *peak* velocities and accelerations can be measured. The disadvantage of this technique is that it only allows assessment of velocities in one part of the myocardium at any one time, and that each section of myocardium must therefore be studied separately, prolonging the examination.

CTDI is more complex. Colour Doppler is overplayed on a grey scale 2d image of the heart and used to colour each pixel of the heart image according to its velocity and direction of motion. Those parts of the heart moving toward the transducer appear red, those moving away appear blue, with brighter hues corresponding to faster velocities. The velocities measured by CTDI are *mean* velocities, unlike the *peak* velocities measured by PWTDI. CDTI allows offline, simultaneous measurement of multiple regions of the myocardium from a single echo loop of just a few cardiac cycles. CTDI has the advantage that data can be collected very quickly at the bedside and later in-depth analysis performed offline. From a single echo loop myocardial velocities can be measured post-acquisition in multiple regions of the heart. However, the ability to collect CTDI data remains limited to more modern and expensive echocardiography machines which can perform the high frame rates required to achieve adequate temporal resolution. This is a particular issue in subjects with higher heart rates and therefore a major theoretical limitation for the use of CTDI in infants.

Although the velocities measured in PWTDI are peak velocities and those in CTDI are mean (and therefore lower), the appearance of the velocity waveforms is similar and highly characteristic. Figure 1.6 below is a schematic representation of the TDI trace produced over one cardiac cycle. In systole there is an initial brief positive deflection which represents isovolumic contraction (IVC) and accompanies the QRS complex of the ECG. This is followed by a prolonged positive deflection (S wave) which corresponds to systolic ejection. After the S wave is a short period of isovolumic relaxation (isovolumic relaxation time, IVRT) followed by two negative

velocities. The first of these is the E' wave, which represents early diastolic filling, and is a measure of myocardial relaxation and chamber stiffness. The second negative velocity is the A' wave which represents late diastolic filling due to atrial contraction, and therefore corresponds to the p wave on the ECG.

Figure 1.6: Schematic diagram of Tissue Doppler waveform



Tissue Doppler waveform over one cardiac cycle (top) with corresponding ECG below.

IVV - isovolumic contraction velocity; **S** -peak systolic myocardial velocity; **IVRT** - isovolumic relaxation time; **E'** - peak early diastolic myocardial velocity; **A'** - peak late diastolic myocardial velocity;

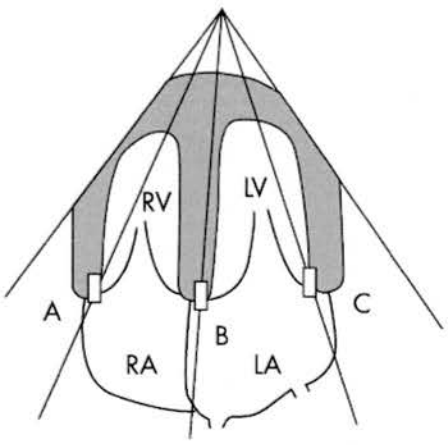
Practicalities of collecting Tissue Doppler Imaging data

Tissue Doppler velocities are typically obtained from an apical view of the heart in the longitudinal plane. Longitudinal motion is less affected by the rotational twisting of the heart during contraction and therefore allows more practicable measurement of

velocities in a single plane. When interpreting TDI velocities in a single plane, however, it must be borne in mind that in some disease processes longitudinal and circumferential fibre function may be differentially affected [78].

From apical views, longitudinal velocities can be measured from any region of the interventricular septum or right and left ventricular free walls. Myocardial velocities decrease from the base of the ventricle towards the apex which is a relatively fixed point at which velocities are low, restricting assessment [78]. The convention in TDI analysis has therefore been to measure velocities at the base of the ventricle where longitudinal movement and velocities are highest. TDI analysis at the mitral valve annulus is used to assess left ventricular function, analysis at tricuspid valve annulus to assess RV function, and analysis of the basal interventricular septum, at the crux of the heart, to assess septal function, as shown in Figure 1.7. Of note, velocities tend to be higher in the RV than the LV [79].

Figure 1.7: Positions for obtaining TDI myocardial velocities



Apical four-chamber view.

A, right ventricular velocities measured at lateral tricuspid valve annulus;

B, septal velocities measured at basal region of interventricular septum;

C, left ventricular velocities measured at lateral mitral valve annulus

As with any Doppler technique the velocities measured are dependent on the angle of insonation of the Doppler beam, according to the equation:

$$\text{Velocity} = \frac{\text{Frequency shift} \times \text{velocity of sound in medium}}{2 \times \text{frequency of transmitted sound} \times \cos\theta}$$

Provided that the angle of insonation (θ) is between 0 and 15 degrees $\cos\theta$ can be assumed to be 1. However, at larger angles of insonation $\cos\theta$ becomes smaller and velocities will be underestimated. This is a limitation of all the Doppler methods described, including TDI.

TDI measurements and their relation to myocardial function

Systolic cardiac function

Three measures of systolic function have been proposed which are measurable from TDI traces; 1) the IVV (isovolumic contraction velocity), 2) systolic ejection velocity (S) and 3) IVA the isovolumic acceleration (i.e. acceleration to reach peak IVV).

The S wave is a prolonged systolic TDI velocity making it easy to identify and measure. Early studies in adults and animals demonstrated a good correlation between S wave velocity in the LV and invasive catheter derived measures of LV function (dP/dT_{max} and maximum elastance; E_{max}) [80, 81]. However Lindqvist et al, studying S velocities in the right ventricle in a pig model found no such relationship

[82]. Furthermore in another pig model RV S wave velocity demonstrated significant load dependence when either preload or afterload was manipulated [49].

In Lindqvist's experiments RV IVV was also measured, and unlike S wave velocity was found to correlate well with dp/dt_{max}, whilst showing no correlation with pulmonary artery pressure. This was interpreted as indicating afterload-independence [82]. However, in their similar pig model Vogel et al demonstrated that RV IVV was significantly affected by changes in load produced by balloon inflation in the pulmonary artery (afterload) and occlusion of the IVC (preload) [49]. However, before discounting IVV as a measure of myocardial function because of its potential load dependence it should be considered that these experiments used extreme loading conditions which may greatly exceed the variations in loading in a disease state. Under more moderate loading conditions, as in Lindqvist et al's study, IVV may still be relatively load-independent and consequently a useful measure of myocardial systolic function.

RV IVA, measured using CTDI, has also been investigated as a measure of contractile performance. In Vogel et al's pig study IVA was the only TDI measure which appeared to demonstrate load independence whilst also correlating well with catheter indices of contractile function during pharmacological manipulations in contractility using dobutamine and esmolol [49]. Pauliks et al have recently reproduced this finding in adults, demonstrating that LV IVA increases in a dose dependent manner with ascending dobutamine dose [83]. However, whilst IVA

appears the most robustly load-independent TDI measure of contractile function there are practical limitations to its use.

Measurement of IVA is best performed using CTDI on the most modern echocardiography equipment, and indeed studies using PWTDI on older echo technology have been unable to measure IVA [82]. Furthermore at higher heart rates the measurement of fast accelerations over very short time intervals becomes increasingly inaccurate and also current technology may not allow fast enough frame rates to measure accurately the rapid changes in velocity.

Diastolic myocardial function assessed by TDI

The diastolic velocities in the TDI waveform are the early diastolic E' velocity and later diastolic A' velocity. TDI E' velocity is determined by myocardial relaxation and passive ventricular compliance whilst A' represents later filling due to atrial contraction [27]. From the TDI waveform, diastolic function can be assessed by measurement of the E' and A' velocities and also by measurement of the isovolumic relaxation time (IVRT) prior to onset of the E' wave.

Experimental studies in adults have shown that the LV E' velocity relates well to an invasive catheter-derived index of myocardial relaxation (Tau; the constant of isovolumic pressure decay) [84]. However, in the LV of the healthy heart E' has also been shown to be significantly preload dependent (i.e. dependent on atrial filling, pressure and atrio-ventricular pressure gradient) [85]. Conveniently, E' appears to be load independent in the failing heart [86].

As discussed earlier, E wave velocities obtained from conventional (non TDI) tricuspid valve Doppler are a composite determined by preload (filling pressures), myocardial relaxation and passive compliance of the ventricle. It has therefore been proposed that calculation of a ratio of E:E' can be used to assess filling pressures (preload). This is supported by adult studies demonstrating good correlation between E:E' and invasive filling pressures in both the right and left sides of the heart [87, 88]. However, other workers have used E:E' ratio in the LV as a measure of the "compliance" properties of the ventricle [89]. Though much is claimed for the utility of the E:E' ratio, it should not be over-interpreted. E:E' is ultimately dependent (like E velocity) on *both* preload *and* compliance but does not indicate the relative contributions of these.

Isovolumic relaxation time (IVRT) provides a different measure of diastolic function which can be measured from TDI velocity waveforms. IVRT is the time from the end of the S velocity to the beginning of the E' velocity and represents predominantly a period of myocardial relaxation but also involves a change in the shape (but not the volume) of the ventricle, adjusting its configuration in preparation for diastolic filling [24, 27]. In addition IVRT is not a pure measure of myocardial relaxation but is also dependent on loading conditions and tricuspid valve competency [90].

In summary TDI E' velocities provide a measure of myocardial relaxation, though with variable preload dependence. E:E' ratio may be used to give a more specific

indication of preload and ventricular compliance but cannot identify the relative contributions of these. IVRT provides additional measures of myocardial relaxation but the former may be load dependent and the latter in particular is open to error in measurement which may be increased at higher heart rates. It is important to note that most of the work to validate these measures has been conducted in animal and adult models and in the LV, rather than the RV. Whether these measures perform in the same way in the RV is relatively unproven.

Use of TDI to assess RV function in pulmonary hypertension and in infants

The use of TDI to assess RV function in pulmonary hypertension has been limited to adult studies. Lindqvist et al have demonstrated a correlation between IVRT and pulmonary artery pressure, using PWTDI in the RV in male adults [91]. These data highlight the load-dependence of IVRT since it is unclear whether the prolonged IVRT was a direct effect of increased afterload and/or a consequence of impaired myocardial relaxation secondary to the increased afterload. Two other groups have compared TDI velocities in adult patients with primary pulmonary hypertension with those of matched normal controls. These studies demonstrated a significant reduction in IVA and S wave velocities in systole and significant reductions in E' wave velocity and E':A' ratio, but not A' wave velocity in diastole [92, 93]. These observations support the findings of both systolic (contractile) and diastolic dysfunction in pulmonary hypertension. A study by Kjaegaard et al in 17 healthy adults in whom hypoxic increases in PVR were induced, also demonstrated significant reductions in S and E wave velocities and TDI IVRT [74]. This group observed no significant differences in IVA, nevertheless the increase in pulmonary

artery pressure in response to hypoxia was relatively small (mean increase in PAP of <10mm Hg). There are no studies using TDI to assess RV function in infants with PHT.

Existing use of TDI in newborn infants has been limited to description of normative TDI velocities. Mori et al demonstrated the feasibility of measuring PWTDI velocities in 130 normal infants within the first 24 hours of life and again in the next seven days. Only systolic S wave velocity and diastolic E'and A' wave velocities were measured however. Velocities were higher in the RV than the LV which in turn was higher than the IVS [94]. TDI has not been employed in disease states in newborn infants, but has been employed antenatally to assess RV function in both fetuses with heart failure and control fetuses. In this study E', A' and S velocities were all seen to increase during fetal life, and E' velocity was significantly reduced in those with heart failure. This finding was interpreted as indicative of diastolic relaxation dysfunction [95] . However, this was a small study with just seven fetuses in the heart failure group and with varied causes of heart failure.

There are no studies reporting use of CTDI in newborn infants either in health or disease. This may be because the level of technology required is not currently available in clinical or research neonatal intensive care settings. Therefore, there is no information on the feasibility of this technique in infants.

Summary of TDI measures

Table 1.2 summarises the discussion of TDI indices and their functional correlates:

Table 1.2: Tissue Doppler Imaging indices and their relation to myocardial function and loading conditions

TDI indices		Myocardial function correlate	Load dependence	Existing infant data
Systolic	IVA	Myocardial contractility	Load independent	No infant data
	IVV	Myocardial contractility	May demonstrate load dependence	No infant data
	S wave	Myocardial contractility	May demonstrate degree of load dependence	Normative data only
Diastolic	E' wave	Myocardial relaxation and ventricular stiffness	Degree of load dependence but may be load independent in heart disease	Normative data only
	A' wave	Atrial contraction and late diastolic filling	Highly preload dependent	Normative data only
	IVRT	Myocardial relaxation and loading conditions	Load dependent	No infant data
	E:E'	Ventricular stiffness and preload (atrial filling pressure)	Highly load dependent	No infant data

In summary TDI is a new form of echocardiographic analysis which makes non-invasive assessment of the components of systolic and diastolic function possible for the first time. This technology remains predominantly a research tool, and has had

very limited reported use in newborn infants. However TDI may be a very useful tool for assessment of infant RV function especially in the setting of pulmonary hypertension. In this thesis the feasibility of both PWTDI and CTDI in normal infants and those with PHT will be investigated, and these techniques used to investigate the mechanisms of RV dysfunction in PHT.

1.4.4.7 Echocardiographic assessment of pulmonary artery pressure

The discussion above has focused on echocardiographic methods of assessing right ventricular function and specifically in the setting of pulmonary hypertension. It is timely at this point to consider also the means of directly assessing PAP, using echocardiography in infants. It is emphasised, however, that pulmonary artery pressure *per se* is a not a measure of RV function, an important issue which is fundamental to this thesis.

Three echocardiographic techniques of assessing PAP are available:

- Tricuspid regurgitation jet velocity
- Ductal shunt assessment
- RV systolic time intervals

Measurement of the velocity of tricuspid valve regurgitant flow (TR) in systole allows calculation of the pressure gradient between RA and RV using a modified Bernoulli formula (Pressure gradient = 4 x $(\text{velocity})^2$). If the pulmonary valve is not stenotic then peak RV pressure equals PAP. This technique demonstrates good

agreement with invasive measurement of pulmonary artery pressure in infants and acceptable repeatability [96] and is now widely employed in current clinical practice. However, it must be noted that frequently TR is not present or an incomplete Doppler trace is obtained which may underestimate PAP: Skinner et al reported that only 19% of term and 31% of preterm infants had pansystolic TR from which peak velocity could be confidently measured [97].

The presence of a patent ductus arteriosus also allows estimation of PAP [98]. Shunting through a patent ductus arterious is dependent on the pressure gradient between the pulmonary artery and descending aorta at any point in the cardiac cycle. Quantification of PA pressures by calculation of the pressure gradient across a patent ductus (using the modified Bernoulli equation) should be cautioned against because peak PA pressure is achieved before peak aortic pressure and therefore the peak systolic pressures do not coincide. Instead, ductal shunting should be interpreted in a still informative, but more qualitative light. Exclusive left to right shunting indicates PA pressures below systemic. Bidirectional shunting of low velocity indicates PA pressures just below or approaching systemic pressures, and exclusive right to left shunting indicates supra-systemic PA pressures.

Other measures of PA pressure are proxy measures based on Doppler-derived RV systolic time intervals. The ratio of time to peak velocity (TPV): RV ejection time (RVET) falls with increasing pulmonary artery pressure and has been shown to correlate with PAP derived from tricuspid regurgitation. However, this technique shows poor repeatability, and furthermore TPV may be variably affected by the

presence of ductal shunting, RV dysfunction and gestational age/body size [99]. In view of these limitations this technique was not used in the work presented in this thesis.

It is important to stress that although these convenient echocardiographic measures provide rapid assessment of pulmonary artery pressure in infants, the pulmonary artery pressure cannot be considered a measure of RV function or vice versa. function and hence illness severity, is highly variable in the face of increased PVR and determined by many factors other than afterload alone.

1.5 Conclusion

Right ventricular dysfunction is an important consequence of pulmonary hypertension in the newborn infant and a principal determinant of disease severity. However, RV function may not be directly related to pulmonary artery pressure alone. Direct assessment of RV function is therefore highly desirable in infants with PHT, but at present no quantifiable measures are routinely available in clinical practice. Furthermore, the mechanisms of RV dysfunction in infants have not been elucidated i.e. the relative contributions of systolic and diastolic dysfunction. Understanding of these may assist in guiding future therapies.

Echocardiographic techniques, including the new developments of tissue Doppler imaging, may allow non-invasive quantification of RV function in infants, and

indicate mechanisms of dysfunction, ultimately allowing better management of these infants.

CHAPTER 2

EXPERIMENTAL DESIGN AND METHODS

2.1 Introduction

The purpose of this chapter is to explain which methods were used in the research and why they were chosen. Initially, the aims of the research and the corresponding testable hypotheses are stated. Next, the process by which these hypotheses were developed into an protocol is discussed. Finally, the experimental design and experimental methods are described in detail.

2.2 Aims and hypotheses

2.2.1 Aims

The previous chapter discussed the importance of assessing RV function in infants with pulmonary hypertension, and potential means of doing so. The overall aim of this research was to identify new methods of assessment and treatment of RV dysfunction in infants with pulmonary hypertension.

At the outset, the fundamental research questions were:

1. Which measures can be used to assess RV function in the infant with pulmonary hypertension?
2. What are the mechanisms of RV dysfunction in pulmonary hypertension?
3. What is the relationship between RV function and pulmonary artery pressure in pulmonary hypertension?

The first question relates to identifying practicable techniques for assessing RV function in infants. This was considered important as current assessment of RV function in clinical practice is limited and without accepted standards. Furthermore, new techniques, not previously investigated in infants, may provide improved assessment of RV function.

The second question seeks to improve understanding of the relative contributions of systolic and diastolic dysfunction to RV dysfunction in infants with PHT. Studies in adults and animals have previously demonstrated both systolic and diastolic RV dysfunction in PHT, but no such studies have been performed in infants [93, 100]. Understanding the mechanisms of RV dysfunction in infants may improve therapy.

The third question seeks to investigate the relationship between RV function and PAP. If RV function is variable in PHT then this emphasises the importance of measuring RV function directly and not solely using the absolute PAP as a measure of illness severity or to predict RV function. Conversely, if the two are linearly related, as studies in animals and older children have suggested [75, 101], then measures of RV function might be used as proxy measures of PAP, for example in those circumstances when a TR jet is absent and PAP cannot be directly measured.

2.2.2 Hypotheses

These experimental aims and research questions were stated as testable hypotheses, (corresponding numerically to the research questions) as follows:

In infants with pulmonary hypertension:

I. RV function can be quantified, in the clinical setting, by non-invasive echocardiographic measures

II. RV dysfunction in PHT is characterised by systolic and diastolic dysfunction

III. RV function is not linearly related to pulmonary artery pressure

2.3 Development of the hypotheses into an experimental design

The fundamental design of this research was the assessment of RV function in infants with pulmonary hypertension and in normal control infants, using a selected number of echocardiographic measures, i.e. a clinical, case-control design. This was intended to allow assessment of these different measures and to elucidate the mechanisms of RV dysfunction in PHT, ultimately to allow more informed clinical therapy.

2.3.1 Overview of experimental design

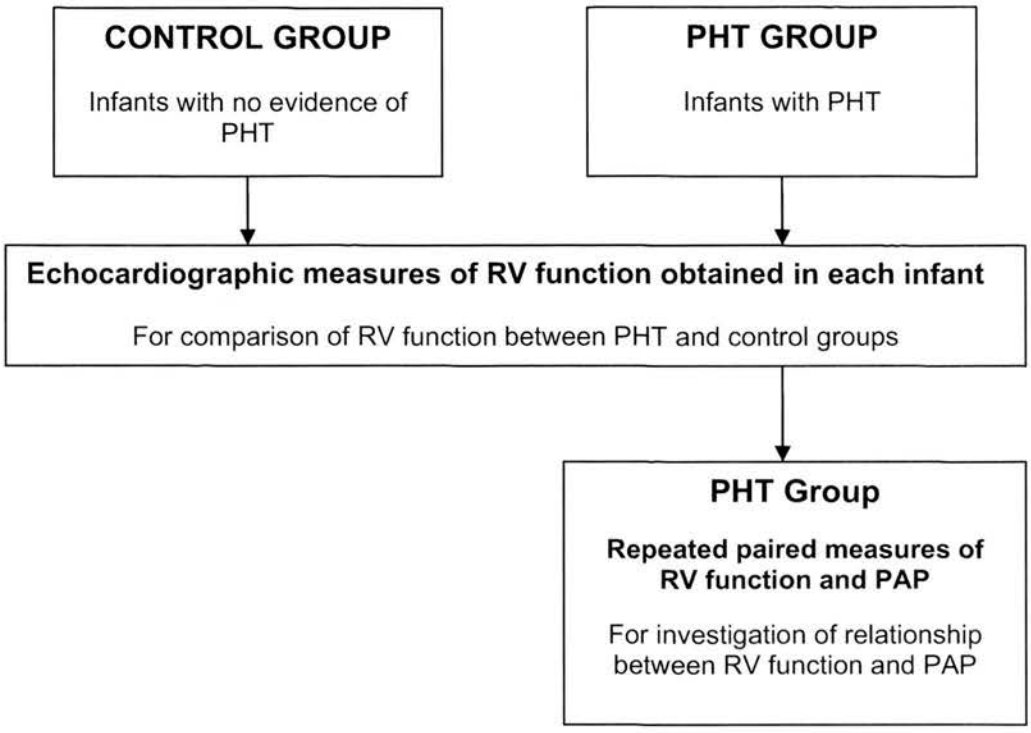
The overall experimental design is summarised in Figure 2.1. Initially, each of the selected measures of RV function was performed in every subject in the control group and each subject in the PHT group. This design allowed assessment of each

pre-selected echocardiographic measure in both normal and PHT infants, thus testing the hypothesis that RV function could be non-invasively assessed by these measures.

The second research question and hypothesis were investigated by comparison of RV function (assessed using the echocardiographic measures) between control and PHT groups to determine the mechanisms of RV dysfunction in the PHT group.

The final hypothesis, that RV function is not linearly related to PHT, was investigated separately by repeated paired measurement of pulmonary artery pressure and RV function (using all of the selected echocardiographic measures of RV function) in infants in the PHT group.

Figure 2.1: Experimental design



The use of a control group provides normative data and a feasibility assessment of the echocardiographic measures of RV function in normal infants. The PHT group allows assessment of the same measures in the disease setting and comparison of RV function in PHT and normal controls, as well as investigation of the mechanisms of dysfunction.

In the PHT group, serial paired measurement of PA pressure and RV function, using all of the selected measures, allowed investigation of the relationship between these two variables.

2.3.2 Use of a human population

Human infants in a clinical neonatal unit setting were selected as the study population. Mechanisms of RV dysfunction in PHT have previously been studied in adults and older children [75, 92, 93]. However, direct extrapolation from these

studies to the infant may not be valid; RV dysfunction may be different in the infant due to the different aetiologies of PHT and the different morphology of the infant RV compared to that of the older child or adult. Animal models, including newborn piglet models, have also been employed to investigate mechanisms of RV dysfunction in PHT, allowing assessment in a controlled laboratory environment [73]. However, inter-species variation may limit extrapolation of these data to the newborn human infant. For these reasons, it was felt preferable to directly study mechanisms of RV dysfunction in human infants with PHT and not in a proxy model, be it an older human or an animal.

As well as investigating the mechanisms or RV dysfunction, this thesis also aimed to investigate new techniques to assess RV function in infants in a clinical setting. Clearly, investigating the feasibility of these techniques for use in infants is best done in a real infant population and not in a controlled animal or adult model.

2.3.3 Importance of a pulmonary hypertension population and control population

A control population of normal infants and a population of infants with PHT were studied in this work. A control population was considered necessary for two reasons: 1) to investigate the feasibility of the new measures of RV function assessment in normal infants, and 2) to provide normative data for each measure of RV function for comparison with data from the PHT group.

2.3.4 Choice of echocardiographic techniques to assess RV function

The techniques selected for assessment of RV function in this study had to be both ethically acceptable for use in sick human infants and, if they are to be recommended for future clinical use, available using currently available technology.

Echocardiography was selected because this technology is non-invasive, can be performed at the cot-side and is already widely used and tolerated in neonatal practice [54]. Invasive conductance catheters represent the "gold standard" for assessment of ventricular function and would ideally have been included in this study both as a means of assessing systolic and diastolic function in PHT and as a means of validating echocardiographic data. However, use of conductance catheters in infants is both technically challenging and associated with serious risks and side effects [50-52]. Cardiac catheterisation was therefore not an ethically acceptable option in this study. The validity of the echocardiographic measures of RV function was instead assumed on the basis of extensive existing data validating these techniques against conductance catheter data in adult and animal studies [49, 82].

The echocardiographic measures selected for study were chosen on their likely ability to meet the criteria for an "ideal measure", i.e. one that can be rapidly obtained from limited echocardiographic views and provides accurate, load-independent, quantification of systolic and diastolic function.

The measures selected were:

- tricuspid valve Doppler inflow velocities
- right ventricular output (RVO)
- myocardial performance index (MPI)

- Tissue Doppler imaging (PWTDI and CTDI)

The relative merits, disadvantages, and current use of each of these techniques are now discussed in relation to their inclusion in this study.

Tricuspid valve Doppler inflow velocities were included in this study because they are well described measures of diastolic function in infants (though not thus far reported in pulmonary hypertension) which can be rapidly obtained from limited echocardiographic views [27].

RVO was included because there has been considerable interest in ventricular output as a practicable clinical measure of cardiac performance in the newborn infant [61, 102]. If the most basic function of the heart is to maintain an adequate cardiac output and systemic perfusion, then it is logical to measure cardiac output (or RVO) as a measure of global cardiac function. However, RVO calculation may be prone to high error [103]. Furthermore, precisely because this is a "global" measure of function, it provides no insight into degrees of systolic or diastolic dysfunction.

Myocardial performance index (MPI) was selected for use in this study as it has the potential to provide rapid quantitative assessment of RV function. MPI has the advantages of being obtained from minimal echocardiographic views, independent of angle of insonation and independent of heart rate [72, 102]. Additionally, adult and animal studies have already shown the utility of this "global" measure of RV function in pulmonary hypertension, though no studies, prior to this one, had been

performed in infants with PHT [73, 104]. Although primarily a research tool, use of MPI in newborn infant disease states has already crept into clinical practice, particularly in Japan (Personal communication, Dr K Suzuki). This represents another reason why proper assessment of MPI in the infant disease setting is now necessary.

Tissue Doppler imaging (both PWTDI and colour TDI) is a relatively new echocardiographic technique, with limited reported use in infants [94, 105]. The inclusion of TDI as a measure of RV function in this study therefore represented an important assessment of this technique in normal infants and infants with pulmonary hypertension. TDI was also selected as a measure of RV function in this study because of its powerful potential to assess directly both systolic and diastolic myocardial function, and therefore determine the contributions of these to myocardial dysfunction in infants with PHT. Importantly, TDI measures of myocardial function, unlike tricuspid valve Doppler and MPI, appear to be relatively load-independent [49, 82].

Both PWTDI and CTDI were used in this study. Though both techniques use Doppler ultrasound to detect low velocity myocardial velocities, they are subtly different [77]. PWTDI is simpler and is widely available on most current cardiac-level ultrasound machines. PWTDI provides real-time, online analysis of myocardial velocities in a single region of myocardium. The main limitations of PWTDI are that separate regions of myocardium cannot be assessed simultaneously, and that the PWTDI has high temporal resolution but poor spatial resolution.

CTDI is more complex and involves colour coding each pixel of myocardium in a 2-dimensional cine-loop. This cine-loop is recorded and then offline TDI analysis performed within any selected region (or regions) of the heart. CTDI therefore allows post-acquisition of any region of the myocardium within the recorded loop and simultaneous TDI analysis in multiple regions of myocardium. CTDI also allows improved spatial resolution compared to PWTDI. It was not clear which, if either, of these techniques was better suited to assessment of RV function in the newborn infant. Both were therefore included.

A number of echocardiographic measures of RV function were not included in this study. Qualitative assessment of RV function, although the most widely used clinical method, is highly observer dependent and provides no objective quantification of ventricular function. The intention of this study was instead to identify a quantitative measure, or measures, of function which could be used to objectively monitor RV function. Geometric measures of ventricular function (ejection fraction and fractional shortening) were not used in this study. Though previously reported in infants [56], these techniques are time consuming and inherently inaccurate due to the complex asymmetrical geometry of the RV. Furthermore, they are load dependent and do not distinguish systolic and diastolic function. Velocity of circumferential fibre shortening (VCF_C) and stress-velocity index (the relation of VCF_C to wall stress) are two further measures of systolic function which have been used to assess left ventricular function in infants [102, 106]. These techniques have

not been attempted, let alone validated, in the infant RV and are arguably too cumbersome for consideration as a practical clinical tool [107].

2.3.5 Assessment of feasibility of RV function measures

An aim of this work, as stated at the beginning of this chapter, was to identify new means of assessing RV function. Accordingly, the hypothesis was generated that new echocardiographic measures could be used to quantify RV function in infants i.e. that the echocardiographic techniques were *feasible*. This assessment of feasibility was important because some of the echocardiographic techniques used in this study (RV_{MPI}, TDI) have rarely been attempted in infants before, and fewer still used in the setting of infant pulmonary hypertension.

Assessment of *feasibility* was based on the following criteria:

- Echocardiographic images, for collection of the raw echocardiographic data, could be practically obtained
- Raw echocardiographic data could be analysed and meaningful measures of RV function calculated
- The technique was well-tolerated by the infant; i.e. there was no clinically significant deterioration in their condition during, or immediately after, collection of echocardiographic data

For each technique feasibility reporting included;

- the proportion of infants in whom echocardiographic data could not be obtained
- the proportion of infants in whom data could not be analysed to generate meaningful measures of RV function
- Consideration of the variability of the measure in the sample population (as summarised by standard deviation)
- the proportion of infants who became distressed or had a clinically significant deterioration during each measure

A clinically significant deterioration was defined as a significant colour change, desaturation or bradycardia. This was based on continuous clinical assessment by the researcher, attending nursing staff and clinicians. At the first indication of clinically significant deterioration, the echocardiogram in progress was interrupted, nursing and attending clinicians alerted, and appropriate measures taken to treat the deterioration e.g. increased inspired oxygen, stimulation.

Of note, feasibility assessment did not include validation of the echocardiographic measures against a "gold standard", nor assessment of the reproducibility or repeatability of the measures. Validation and acceptable reproducibility are important requirements for a clinical measure. As previously discussed, however, validation of echocardiographic measures against the "gold standard" conductance catheter method, is not ethically acceptable in infants. Also, it was not practicable to make assessments of reproducibility, because of limited time and subject numbers.

2.3.6 Measurement of pulmonary artery pressure

Assessment of pulmonary artery pressure was a pre-requisite in infants to be included in the PHT group in this study. This is most accurately done invasively using pulmonary artery catheters but insertion of catheters for this purpose, with the attendant risks, would have been unethical in the study population. Instead, PAP was estimated non-invasively using the Doppler echocardiographic techniques of tricuspid valve regurgitation velocity and patterns of shunting through a patent ductus arteriosus (when present) [96, 98].

2.4 Recruitment of Control and PHT groups

2.4.1 Site of study

All subjects were recruited from the inpatient population of the Neonatal Unit (NNU) of the Royal Children's Hospital (RCH), Melbourne, Australia. Patients managed in this unit are *ex utero* transfers, referred within the neonatal period from throughout Victoria and interstate Australia for management of surgical neonatal problems, respiratory, cardiac, renal, metabolic, infective and neurological diseases, in both term and preterm infants. The Neonatal Unit at RCH has particular specialist experience in the management of congenital diaphragmatic hernia, severe respiratory disease requiring high frequency oscillatory ventilation, and intra-cranial arterio-venous malformations (vein of Galen malformations).

Recruitment of patients for echocardiographic assessment of ventricular function was approved by the Research and Ethics Committee of the Royal Children's Hospital. Infants were recruited between March 2006 and May 2007.

2.4.2 Control group - Eligibility Criteria

Infants were eligible for inclusion in the control group if they:

1. Were inpatients in the Neonatal Unit
2. Were haemodynamically stable and not receiving any cardiovascular medications
3. Were in sinus rhythm

Infants were excluded from the control group if:

1. They had evidence of structural or functional cardiac disease (on clinical or echocardiographic assessment)
2. They had evidence of pulmonary hypertension on clinical or echocardiographic assessment
3. The were too unstable to tolerate echocardiographic examination without clinically significant deterioration, as judged by the attending clinician, nursing staff or study researcher
4. An echocardiogram could not be practicablely performed; e.g. presence of surgical wound preventing access to chest.
5. They had previously been included in the study

2.4.3 PHT Group - Eligibility Criteria

Infants were eligible for inclusion in the PHT group if:

1. They were inpatients in the NNU of the Royal Children's Hospital, Melbourne
2. They had echocardiographic evidence of pulmonary hypertension
3. They were in sinus rhythm

Infants were excluded from the PHT group if:

1. They had structural heart disease (not including a patent ductus arteriosus or patent foramen ovale)
2. The were too unstable to tolerate echocardiographic examination without clinically significant deterioration, as judged by the attending clinician, nursing staff or study researcher
3. An echocardiogram could not be practicablely performed; e.g. presence of surgical wound preventing access to chest.

For the purposes of inclusion criteria, pulmonary hypertension was defined as peak pulmonary artery pressure of at least two thirds peak systolic systemic pressure when estimated from tricuspid regurgitation jet, or the presence of significant right-to-left shunting through the patent ductus arteriosus (i.e. bidirectional ductal shunting or exclusive right-to-left ductal flow). Measurement of pulmonary artery pressure using these techniques is discussed below. If an infant was suspected of having pulmonary hypertension but pulmonary artery pressures could not be assessed by

echocardiography (i.e. no ductal shunt or tricuspid regurgitation jet), then they were not recruited to the study.

Infants with patent arterial ducts and/or patent foramen ovale were not excluded from the PHT group. Patent arterial ducts and patent foramen ovale are relatively common findings in the first days of life. In infants with pulmonary hypertension and right-to-left shunting, ductal patency may be prolonged. In addition, in some infants with severe PHT, prostaglandin E1 may be utilised to maintain ductal patency and allow right-to-left ductal shunting as a means of reducing afterload on the pressure and volume loaded RV. This study did not seek to distinguish the effects of ductal and foramen ovale patency on RV function and therefore did not exclude infants on the presence or absence of these shunts, although their presence and associated shunting were noted.

Infants with all causes of PHT were recruited. The limited and unpredictable numbers of infants admitted to the NNU with PHT meant that it was not practicalable to limit the PHT group to infants with a single diagnosis.

2.4.4 Experimental conditions

All experiments were conducted within the Neonatal Unit. Echocardiography was performed at the subject's cot-side. Subjects were studied when they were settled or sleeping. Those infants who were feeding enterally were studied approximately 30 minutes after a feed. To pacify those infants who were awake during the experiments, up to 0.5 mL of a 33% sucrose solution was given orally. This was in accordance with protocols for clinical management of infants in the NNU. A number of enrolled infants were already sedated and muscle relaxed for clinical reasons, which assisted echocardiographic study. Infants were positioned in a flat, supine position for all echocardiographic studies. ECG electrodes, transcutaneous CO_2 probes and temperature probes were moved to allow access to the chest and upper abdomen for echocardiography.

Most experiments were conducted in the evenings and at weekends, as this was the only time when access to the echocardiography machine was available. Conveniently, this was also the time when the NNU was quietest and infants were less likely to be disturbed by environmental noise and activity, or for clinical purposes. No attempt was made by the researcher to alter the clinical management of any study subject prior to or during the study.

2.5 Demographic and therapeutic data

For each subject the following demographic data were recorded from the clinical notes:

- gestation
- age
- corrected gestational age
- weight
- sex
- diagnosis

Data were also obtained on the clinical treatments that each subject was receiving, specifically:

- Ventilation or respiratory support i.e. use of nasal prong oxygen, continuous positive airway pressure (CPAP), conventional ventilation, high frequency jet ventilation (HFJV) and high frequency oscillatory ventilation (HFOV)
- Inspired oxygen concentration
- Cardiovascular drugs i.e. dopamine, dobutamine, milrinone, adrenaline, noradrenaline
- Inhaled nitric oxide
- Prostaglandin E1 infusion (employed to maintain ductal patency)

2.6 Physiological data

A three lead electrocardiogram (ECG) was recorded simultaneously with all echocardiographic data (Philips IE33, Philips, Bothwell, WA). ECG gain was adjusted (typically 60-75%) to ensure that distinct P waves and QRS complexes were identifiable. An adequate ECG recording was essential for determination of cardiac cycle phases on echocardiograms. This was particularly important when identifying myocardial velocities in tissue Doppler imaging. Heart rate was calculated automatically from the ECG and monitored continuously.

Systemic blood pressure (BP) was recorded in all infants in the PHT group at the beginning of each study. BP data were required for comparison with pulmonary artery pressure. BP was obtained from the bedside clinical monitor (Philips Intellivue 80/90, Philips, Bothwell, WA) using a cuff sphygmomanometer or invasive arterial catheter if one was already in use for clinical monitoring (radial, posterior tibial or umbilical artery catheter).

Transcutaneous oxygen saturation (SaO_2) was monitored for the study duration if it was already in use for clinical monitoring of the patient. SaO_2 data provided additional information on the safety and tolerability of different echocardiographic measures.

2.7 Echocardiographic data acquisition

All echocardiographic measures were made using a Philips IE33 (Philips Medical, Bothell, WA), with an 8 MHz probe. All echocardiograms were performed by the study researcher (NP), who trained formally in echocardiography as a Specialist Trainee in paediatric cardiology, and is an extensive user of echocardiography in the clinical neonatal setting. NP had been previously trained in the use of TDI by a supervising Paediatric Cardiologist (Dr Michael Cheung) who has extensive clinical and research experience with this technique.

An initial "screening" echocardiogram was performed in each infant to identify structural heart disease which would exclude the infant from the study. This echocardiogram typically took approximately 20 minutes. If an abnormality was detected on the screening echocardiogram then the attending neonatal Consultant was informed and referral made to the Paediatric Cardiology department for further assessment. Confirmation of structural heart disease excluded the infant from the study. If an infant had a previously documented structural echocardiogram performed for clinical purposes by the study researcher (NP) or another experienced echocardiographer (Paediatric Cardiology Fellow or Consultant) then no screening echocardiogram was performed.

After the initial screening examination the following echocardiographic data were obtained:

- Tricuspid valve Doppler (pulse wave and continuous wave Doppler)
- Pulmonary valve pulse wave Doppler
- 2-dimensional image of right ventricular outflow tract including pulmonary valve
- 2-dimensional image of patent ductus arteriosus (when present) and ductal flow pulse wave Doppler
- CTDI data acquisition
- PWTDI data acquisition

These raw data were used to obtain the measures of RV function: tricuspid valve inflow velocities, MPI and RVO, CTDI and PWTDI myocardial velocities, and estimation of pulmonary artery pressure.

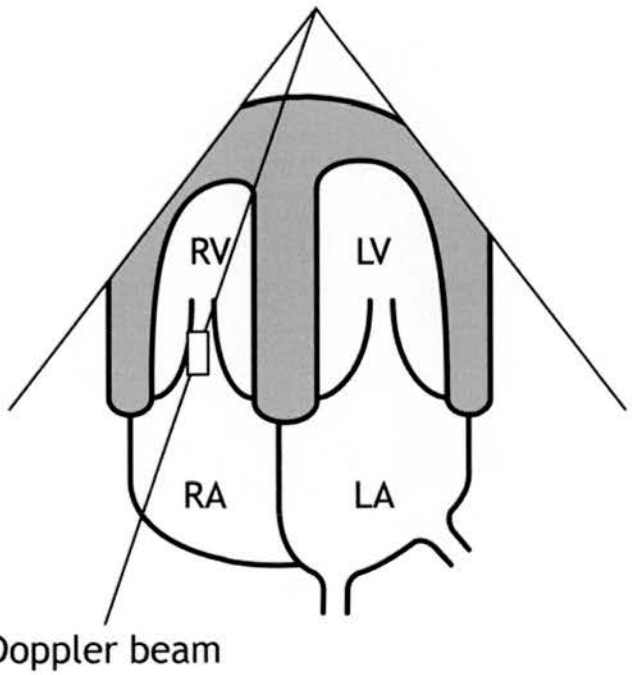
In collecting echocardiographic data, consideration was given to any displacement of the heart from the normal position. This was a particular issue in infants with congenital diaphragmatic hernia in whom the heart is usually displaced towards the contralateral side, away from the diaphragmatic defect and herniating abdominal contents.

All echocardiographic data were obtained over five cardiac cycles and a mean obtained. All Doppler data was recorded from a minimal angle of insonation ($<15^{o}$). No angle correction was made.

2.7.1 Tricuspid valve pulse wave Doppler and continuous wave Doppler

The tricuspid valve was imaged from a 4-chamber view obtained from an apical position (i.e. below the left nipple in normal infants, or more medially in infants with left congenital diaphragmatic hernia). Colour Doppler was used to visualise blood flow and then the Doppler sample volume was placed in the centre of the colour flow, between the valve tips, as demonstrated in Figure 2.2. A pulse wave Doppler waveform was obtained at this position, for offline measurement of inflow velocities. These data were required for measurement of tricuspid valve inflow velocities (E and A wave velocities) and calculation of MPI.

Figure 2.2: Apical 4 chamber view for tricuspid valve Doppler recording



Doppler sample volume positioned between valve tips of tricuspid valve. Care taken to minimise angle of insonation to <15°

Colour Doppler was then used to identify any tricuspid regurgitation (TR) jet. If TR was present then continuous wave Doppler was used to record the velocity along a plane through the middle of the TR jet. This data was required for assessment of pulmonary artery pressures using TR jet velocity.

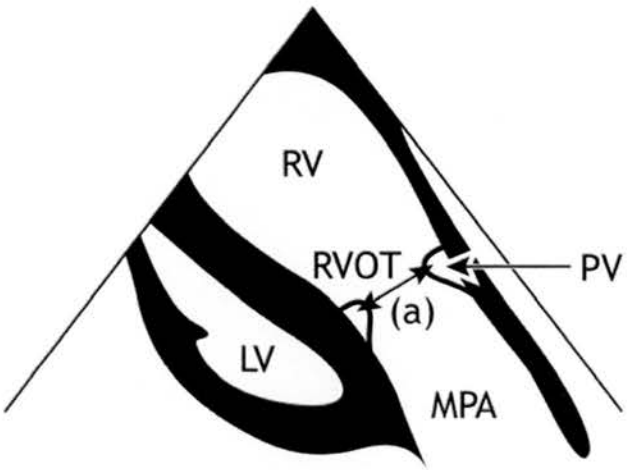
Pulse wave Doppler was used for assessment of inflow velocities because the technology allows assessment of low velocities within a small sample volume, excluding extraneous blood flow velocities along the axis of the Doppler beam. However, pulse wave Doppler is unable to measure the higher velocities seen in TR jets in pulmonary hypertension. Instead, continuous wave Doppler was used to assess these higher velocities, but had the disadvantage that the recorded Doppler waveform was a composite of all velocities along the entire plane of Doppler beam.

2.7.2 Pulmonary valve pulse wave Doppler

Pulmonary valve pulse wave Doppler data was required to calculate RVO and MPI. The pulmonary valve was visualised using 2-d echocardiography from a left parasternal long axis view as shown in Figure 2.3. This position was displaced medially in infants with left-sided congenital diaphragmatic hernia. In each infant the image was optimised, by depth and angle adjustment, to ensure that the pulmonary valve was positioned centrally in the echo window (as seen in Figure 2.3). Colour Doppler was then used to visualise blood flow across the pulmonary valve. The pulse wave Doppler sample was then positioned within the midstream at the

level of the pulmonary valve leaflet tips and the Doppler velocity waveform obtained.

Figure 2.3: Parasternal long axis view of pulmonary valve for pulse wave Doppler recording and pulmonary valve diameter measurement



LV, left ventricle; **RV,** right ventricle, **RVOT**, right ventricular outflow tract; **PV**, pulmonary valve; **MPA**, main pulmonary artery. Pulse wave Doppler sample placed between pulmonary valve leaflets. Measurement "a" is the valve diameter measured between hinge points on the 2-dimensional image.

2.7.3 Pulmonary valve diameter

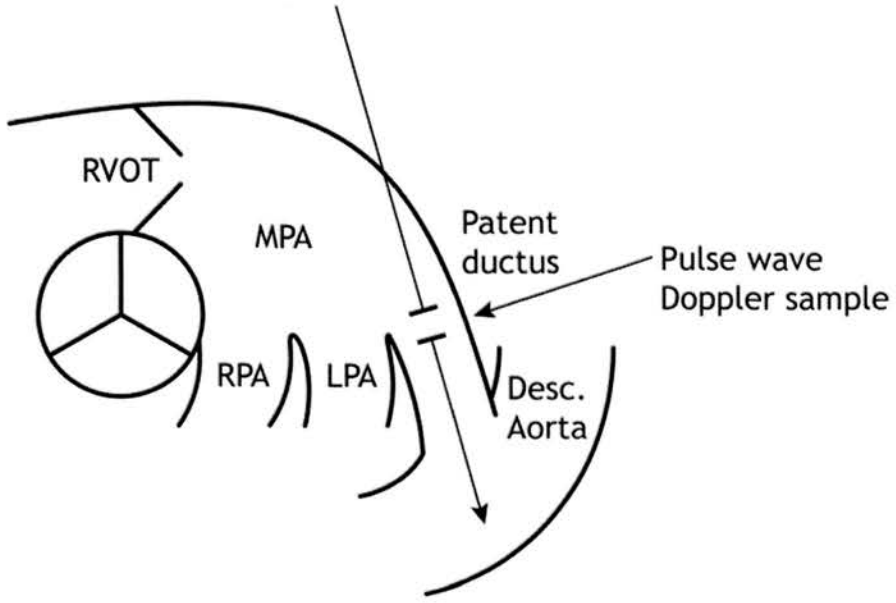
Pulmonary valve diameter was required for calculation of RVO, and was measured from a 2-dimensional view of the valve obtained from a parasternal long-axis view. Valve diameter was measured at the hinge points of the valve, at end systole using frame-by-frame analysis (see Figure 9). This measurement was repeated over five consecutive cardiac cycles, to reduce measurement error. The decision to measure

valve diameter at the hinge point was based on a previous study which demonstrated the highest inter-user repeatability for measurements at this position, compared to measurements of pulmonary trunk and right ventricular outflow tract diameters [65].

2.7.4 Imaging and Doppler flow of patent ductus arteriosus

Two-dimensional echocardiography, with the addition of colour Doppler, was used to identify the presence or absence of flow in a patent ductus from a high left parasternal (ductal) imaging window. This technique of assessing ductal flow has been previously well described and is accepted clinical practice [55, 108-110] . Maximum ductal diameter was measured, from the colour Doppler flow image, at the confluence of the patent ductus and the main pulmonary artery, using frame-by-frame analysis. Pulse wave Doppler of ductal flow was obtained by positioning the Doppler sample in the ductus at the same confluence of ductus and pulmonary artery (Figure 2.4). If ductal flow velocity exceeded the limits of the pulse wave scale (>1.5m/sec) then continuous wave Doppler was used instead. Ductal Doppler flow recordings were required for determination of pulmonary artery pressures.

Figure 2.4: Ductal view and position for Doppler recording of ductal flow



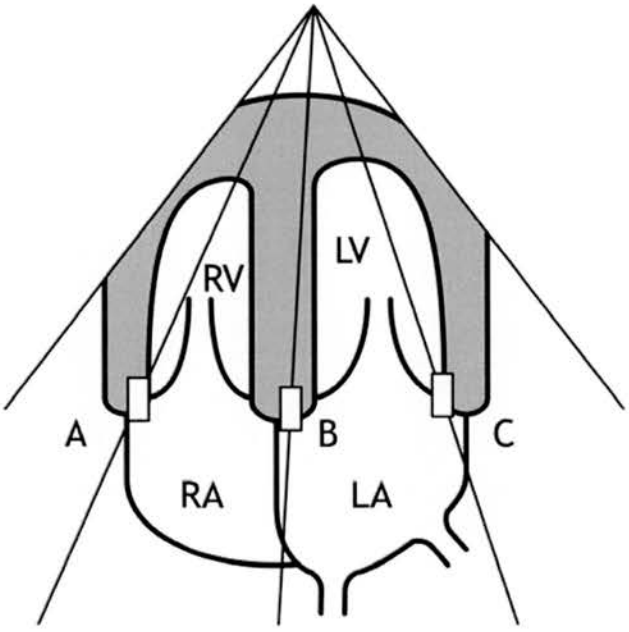
RVOT, right ventricular outflow tract; **MPA**, main pulmonary artery; **RPA**, right (branch) pulmonary artery; **LPA**, left (branch) pulmonary artery.

2.7.5 Pulse wave TDI

In the 2-dimensional apical four chamber view, pulse wave Doppler was selected and the Doppler sample was positioned in the myocardial region of interest. The smallest TDI sample volume size was used (2 mm). TDI was then selected, producing a waveform of myocardial velocities against time, from the region of myocardium within the Doppler sample. The velocity scale was adjusted to produce an optimum Doppler waveform (typically scale <10 cm/sec) and the sweep speed set to maximum (150 mm/sec). TDI gain and reject settings were adjusted to produce the highest resolution image avoiding blurring of the waveform and "speckling". Blurred waveforms, secondary to excessive gain, may lead to overestimation of peak myocardial velocities.

PWTDI was acquired in real time at the following positions; the basal right ventricle at the lateral tricuspid valve annulus, the basal interventricular septum (IVS) at the insertion of the mitral and tricuspid valves, and the basal left ventricle at the lateral mitral valve annulus (Figure 2.5). Raw PWTDI data was used for measurement of myocardial velocities and diastolic time intervals.

Figure 2.5: Positions for acquisition of PWTDI data from apical 4 chamber view

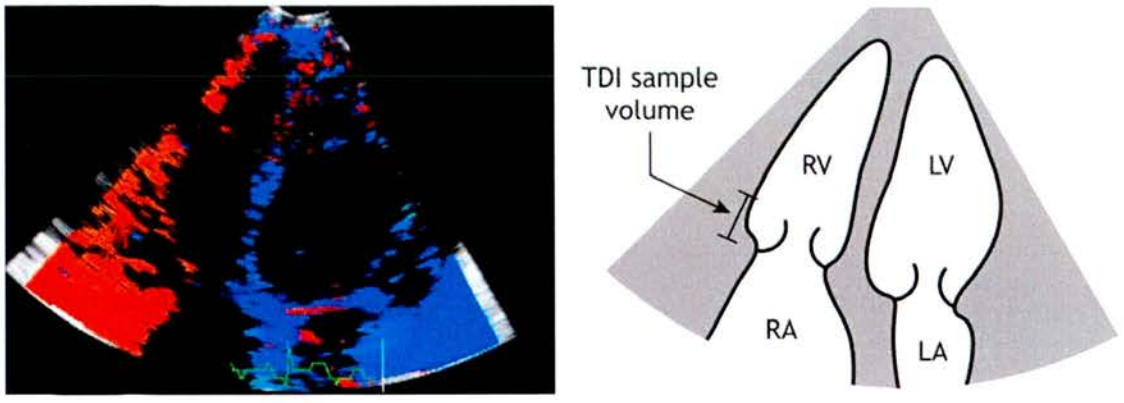


RA, right atrium; **RV**, right ventricle; **LA**, left atrium; **LV**, left ventricle.
A – basal right ventricle position, position for collection of RV TDI data
B – basal interventricular septum, position for collection of IVS TDI data
C – basal left ventricle, position for collection of LV TDI data

2.7.6 Colour TDI loop acquisition

An optimal apical four chamber view of the heart was obtained using 2-dimensional echocardiography and then colour TDI mode selected. The velocity scale was adjusted to select out myocardial velocities (<10 cm/sec) and colour gain was adjusted to achieve good colour-coding of the myocardium, without extraneous colorization of blood flows within the heart (Figure 2.6). A colour coded TDI cine-loop of six consecutive cardiac cycles was then stored digitally for offline analysis of CDTI myocardial velocities.

Figure 2.6: Apical 4 chamber view before and after colour coding with CTDI



Left image: 2-dimensional apical four chamber view, with colour TDI mode selected
Right image: Corresponding schematic diagram of apical four chamber view. TDI sample volume in basal RV position for collection of RV TDI data

2.8 Post-acquisition analysis of echocardiographic measures of RV function

From the raw echocardiographic data, the following measures of RV function were obtained by post-acquisition analysis:

- Tricuspid valve inflow velocities
- Right ventricular output
- Myocardial performance index
- PWTDI myocardial velocities and diastolic time intervals
- CTDI myocardial velocities

All measurements of Doppler velocities and time intervals were repeated over five consecutive cardiac cycles, to take into account physiological variation including the influence of respiratory variation.

2.8.1 Tricuspid valve inflow velocities

Peak E and A wave velocities were measured manually from the pulse wave Doppler waveform of tricuspid inflow, using inbuilt analysis software on the Philips IE33. If no E wave could be clearly distinguished then a velocity of "0" was recorded.

2.8.2 Right ventricular output (RVO)

RVO was calculated using the flow calculation:

$$\text{RVO} = \frac{\textit{velocity-time integer (VTI)} \times \textit{heart rate} \times \textit{cross sectional area of pulmonary valve}}{\textit{weight}}$$

The units of VTI were cm, of heart rate were beats-per-minute, and of CSA were cm^2. RVO was expressed in mL/kg/min.

Velocity time integer (VTI) was obtained from the pulmonary valve pulse wave Doppler flow waveform. Using the trace function of the analysis software on the IE33, each Doppler flow (one cardiac beat) was manually traced out and the area under the traced curve automatically calculated. The area under the curve is the VTI, which represents the distance travelled by a column of blood, across the valve, in each heartbeat. The heart rate was automatically calculated from the accompanying ECG.

The cross sectional area (CSA_{PV}) of the pulmonary valve was obtained using the following formula:

$$CSA_{PV} = \pi \, (\text{valve diameter})^2 \, / \, 4$$

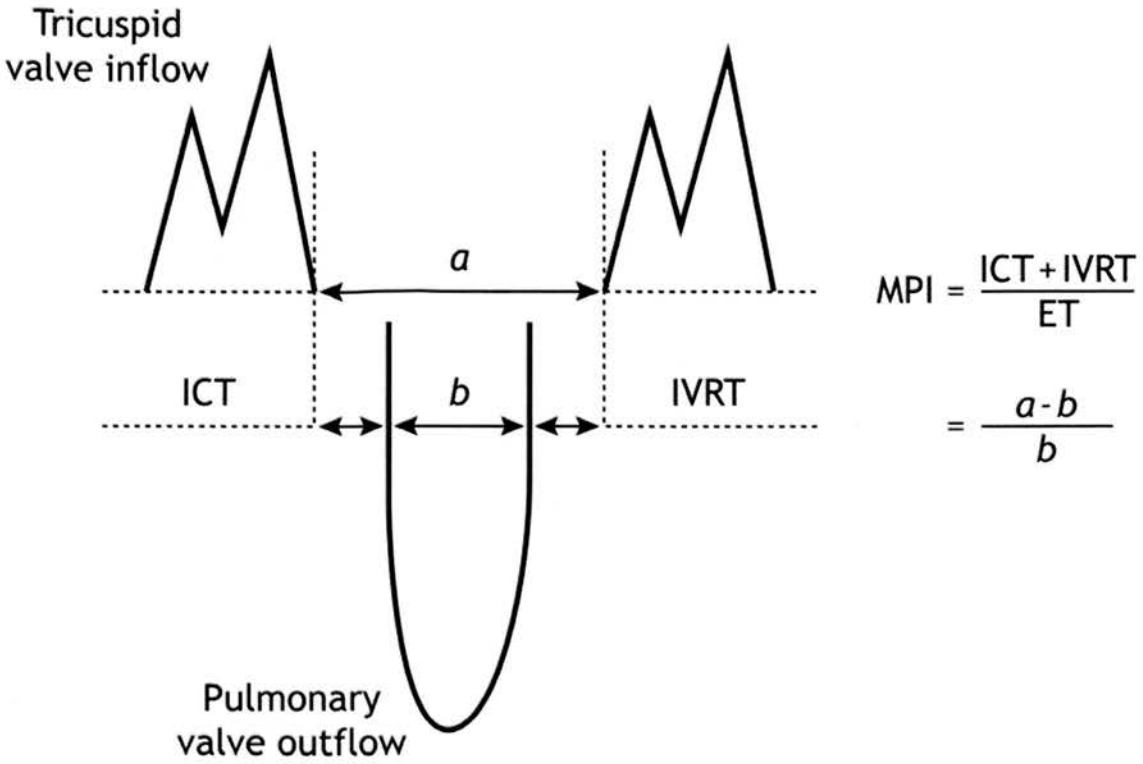
The valve diameter was obtained by measurement from the two-dimensional, long axis image.

Calculation of RVO in this way is liable to significant error [103]. Error present in any of the three variables (VTI, valve diameter and heart rate) is multiplied when inserted into the formula to calculate RVO. Furthermore any error in measurement of valve diameter is increased by squaring this measurement to obtain CSA.

2.8.3 Myocardial performance index

RV_{MPI} is the sum of RV isovolumic contraction (IVCT) and isovolumic relaxation time (IVRT), divided by the RV ejection time (RVET). RV_{MPI} was calculated from tricuspid valve inflow and pulmonary valve outflow pulse wave Dopplers, (Figure 2.7) as has previously been described by Ishii et al [111]. The time period from cessation to beginning of tricuspid inflow (a) and the right ventricular ejection time (b) were measured. RV_{MPI} is equal to (a-b)/b. One limitation of RV_{MPI} calculated in this way is that heart rate (and therefore IVRT and ICT) may vary between the tricuspid and pulmonary valve Dopplers. Care was therefore taken to select paired pulmonary and tricuspid Doppler flows between which R-R intervals varied by less than 5 beats per minute.

Figure 2.7: Calculation of RV_{MPI}



a – time interval cessation to beginning of consecutive tricuspid valve inflow

b – right ventricular ejection time

ICT, Isovolumic contraction time; IVRT, Isovolumic relaxation time

2.8.4 PWTDI data analysis

Post acquisition analysis was performed on each PWTDI waveform to obtain the following velocities and time intervals:

Systolic velocities:

- Isovolumic velocity (IVV)
- Systolic ejection velocity (S)

Diastolic velocities:

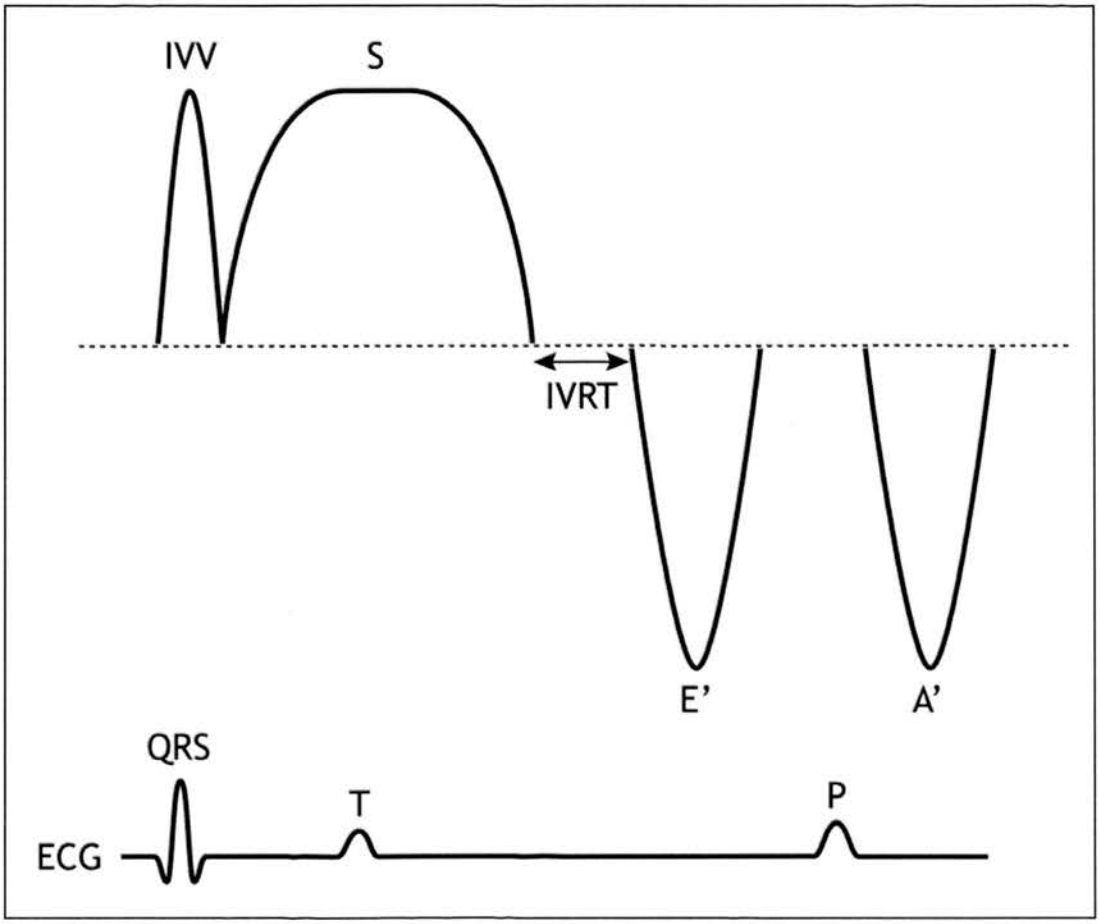
- Early diastolic velocity (E')
- Late diastolic velocity (A')

Time intervals:

- (Diastolic) Isovolumic relaxation time (IVRT)
- Diastolic filling time (DMDT)
- R-R interval

These velocities and time intervals are shown in Figure 2.8 below. All velocities and time intervals were measured from five consecutive cardiac cycles and means obtained.

Figure 2.8: Schematic diagram of PWTDI waveform with corresponding ECG



IVV, systolic isovolumic contraction velocity; **S**, systolic ejection velocity; **E'**, early diastolic velocity; **A'**, late diastolic velocity (occurs after P wave on ECG); **IVRT**, isovolumic relaxation time. **DMDT** (not indicated) is the time from beginning of E' to cessation of A' velocities.

Velocities and time intervals were measured manually using the measurement software on the Philips IE33. Maximal velocities in the PWTDI waveform were measured. Specific velocities were identified by their characteristic appearance and their timing in relationship to the ECG. IVV is a brief positive deflection corresponding to the ECG Q wave. S occurs after IVV and is a prolonged systolic velocity beginning at the ECG R wave. The E' wave is a negative deflection after

the S wave and before the P wave of the ECG. The A' wave is a negative deflection immediately following the P wave of the ECG. Distinguishing these components of the PWTDI waveform was important in the setting of abnormal myocardial function where the velocities may be significantly altered from the normal pattern and care was taken to identify velocities correctly.

IVRT was measured from the cessation of the S wave to the beginning of the E' wave. However, based on the researcher's experience, it was predicted that in some infants IVRT may be difficult to measure accurately. Specifically it was felt that the combination of high infant heart rates, short IVRT and spectral broadening of the PWTDI waveform may introduce error into this measurement.

Given these concerns of inaccurate IVRT measurement, it was also decided to measure another time interval: diastolic filling time (DMDT). DMDT has not been reported before and was a new measurement. DMDT was defined as the period from onset of the E' wave to cessation of A' wave. These time points are easily identifiable allowing more reliable measurement of DMDT than IVRT. DMDT represents the period during which there is diastolic displacement of the myocardium associated with ventricular filling. DMDT was employed as a reciprocal measure of IVRT: this makes the assumption that changes in IVRT produce reciprocal inverse changes in DMDT i.e. lengthening of IVRT will produce a shortening of DMDT. This assumption is not supported by any prior evidence, a limitation which is discussed in interpretation of the diastolic time interval data later in this work.

R-R interval was measured from the beginning of one IVV to the beginning of the next IVV. R-R interval was measured so that IVRT and DMDT could be "corrected" for heart-rate by calculation of IVRT:R-R ratio and DMDT:R-R ratio. This correction was intended to allow comparison of these time intervals between infants with different heart rates. The validity of this correction was tested by investigation of the relationship between IVRT and R-R interval, and the relationship between DMDT and R-R interval.

2.8.5 Colour Tissue Doppler Imaging Analysis

Post acquisition analysis of TDI data was performed using Philips Qlab software operating on the Philips IE33. Digitally recorded colour-coded TDI cine-loops, obtained in the four-chamber view over five consecutive heart beats were uploaded for analysis.

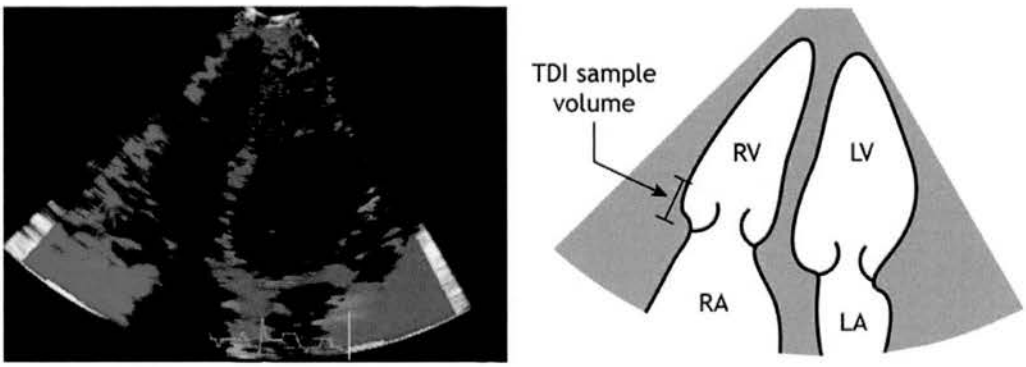
The myocardial region of interest for analysis was selected from the four-chamber view by manually drawing a sample volume ("M-line"). The sample volume was drawn along the longitudinal plane of the myocardium and adjusted to a standard length of 5 mm and width of 2.5 mm. The cine CTDI loop was then played at low speed and the sample volume's position manually adjusted throughout each cardiac cycle to maintain position within the myocardial region of interest. Care was taken to position the sample volume within the myocardium and not in the epicardium, pericardium or within the cardiac chambers.

The sample volume was positioned in the basal RV at the lateral tricuspid annulus to assess RV velocities, and at the basal interventricular septum at the medial insertions of the tricuspid and mitral valves to assess interventricular septum velocities. These were the same positions in which PWTDI velocities were obtained.

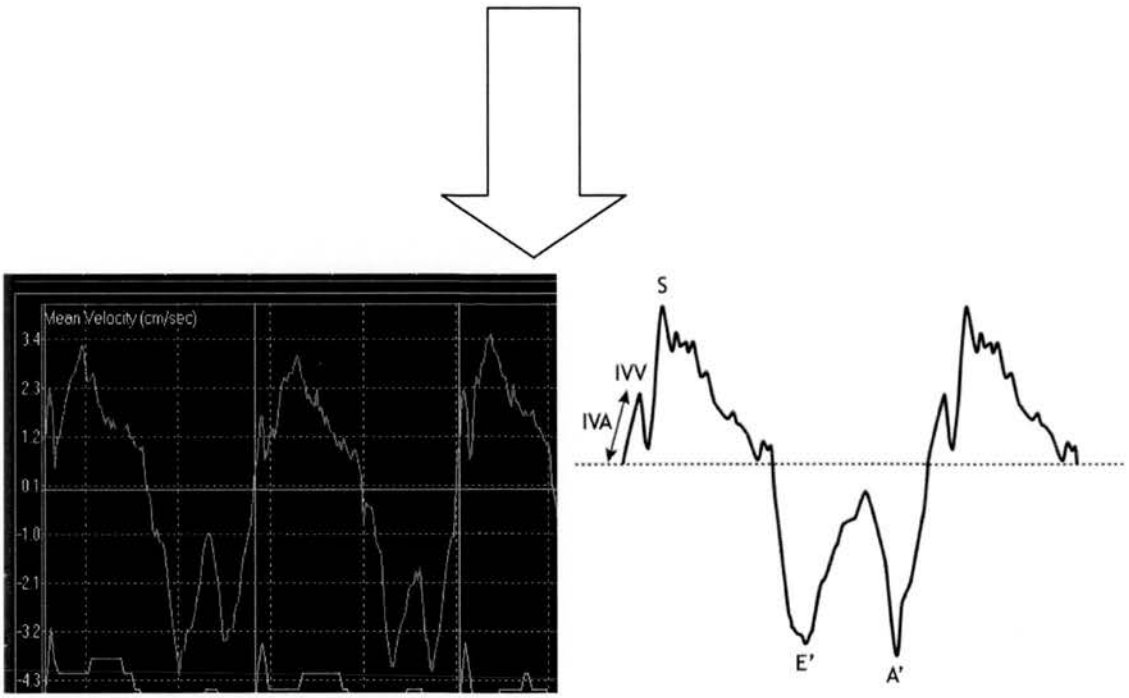
CTDI analysis of myocardial velocities was performed at frame rates of 189-212 Hz, i.e. within the sample volume the myocardial velocity was measured 189-212 times per minute. The CTDI waveform produced is a graph of the average velocity within the sample volume, against time, over the duration of the recorded echo loop (i.e five cardiac cycles in this study). Figure 2.9 is a representative CTDI waveform produced in this way.

Curve processing was not used in analysis of the CTDI data. This functionality of the processing software allows "smoothing" of the CTDI waveform but may lead to loss of short-lived velocities within the waveform, such as IVV.

Figure 2.9: Analysis of CTDI data to produce myocardial velocity waveform



Four chamber apical view in CTDI mode (left) and corresponding schematic diagram (right). CTDI sample volume positioned in right basal myocardial region. Analysis of myocardial velocities in this region using Qlab software generates velocity waveform as seen below.



Sample CTDI myocardial velocity waveform (left) and corresponding schematic diagram (right). IVA, isovolumic acceleration; IVV, isovolumic contraction velocity; S, systolic ejection velocity; E', early diastolic velocity; A', late diastolic velocity.

IVV, S, E and A waves were identified from their appearance and timing in relation to ECG data, as was similarly described for PWTDI velocities. CTDI velocities were manually measured using Qlab software. Isovolumic acceleration (IVA) was also measured. IVA has previously been reported as a load-independent measure of systolic ventricular function [49]. IVA was calculated by positioning the measuring caliper at the peak A' wave velocity and adjacent peak IVV velocity. The slope of the line between these two points represents IVA.

Time intervals (IVRT, DMDT) were not measured from the CTDI waveform. Following initial trial attempts to measure these intervals on CTDI, it was realised that the Qlab software restricted measurement of time intervals to those between fixed frames, or sample points, which in turn were dependent on the frame rate. Sample points could not be manually positioned at the preferred positions at which time intervals would ideally have been measured. It was therefore decided not to measure time intervals from CTDI data.

2.9 Estimation of pulmonary artery pressure

Pulmonary artery pressure was estimated using two techniques, 1) velocity of tricuspid regurgitation (TR) and 2) pattern of ductal shunting.

If TR was present then a maximal velocity was measured from the continuous wave Doppler waveform of the tricuspid valve regurgitant jet flow. A modified Bernoulli

equation was then used to calculate the pressure gradient between the right atrium and right ventricle [96]:

$$\textbf{\textit{Pressure gradient = 4 x (maximal TR velocity)}}^2$$

Pulmonary artery pressure was quantified by adding 5 mmHg (an estimation of right atrial pressure) to this gradient. PAP calculated in this way was divided by systemic systolic BP and was classified as greater than two-thirds systemic (but less than systemic), systemic or supra-systemic. PAP was only estimated in this way if TR Doppler waveform had been obtained from which peak TR velocity could be clearly obtained. An incomplete waveform with an indistinct maximal velocity was not considered suitable for calculation of PAP pressure.

PAP pressure was also estimated by assessment of ductal shunting [55, 97]. The pulse wave Doppler waveform of ductal flow was assessed visually. If shunting was exclusively, or predominantly left-to-right, but of low velocity (<2 m/sec), then this was considered to demonstrate sub-systemic PHT. If shunting was bidirectional and of low velocity (<2m/sec), then this was considered to demonstrate systemic PAP. If ductal shunting was exclusively or predominantly right to left, this was considered to demonstrate supra-systemic PAP.

No attempt was made to quantify peak PAP from ductal shunt velocities. Although a ductal pressure gradient may theortetically be calculated from ductal shunt velocities using a modified Bernoulli equation, this cannot be used to accurately determine

PAP without corresponding knowledge of the timing of peak systolic pulmonary artery and systemic pressures [55].

Repeated measures of all RV function data and PAP were made in the PHT group to investigate the relationship of PAP and RV function. In these studies, it was important to quantify PAP and therefore only the TR jet velocity method was used. If no TR jet was present, or the maximal TR velocity could not be clearly identified on the Doppler waveform, then the study was not included in later analysis.

2.9 Data storage

All raw echocardiographic data was saved in digital format to recordable digital versatile disc (Sony DVD-R, 8x, 4.7GB; Sony Corporation; Tokyo, Japan). Control studies were randomly assigned a unique three digit code to anonymise data. Studies were uploaded from DVD to the IE33 for subsequent analysis.

2.10 Statistical analysis

All demographic data and treatment data obtained from the clinical notes, were recorded in a pre-formatted spreadsheet (Microsoft Excel; Microsoft Corporation, Seattle, WA, USA). All echocardiographic data, recorded from five consecutive cardiac cycles, were entered manually into a separate spreadsheet for each subject. Subject means were combined to generate separate control and PHT group datasets.

The proportion of infants in whom echocardiographic data could not be acquired or analysed, was calculated for each measure of RV function.

Group data were tested for normality (Gaussian distribution) using a Kolmogorov-Smirnov test. Continuous, normally distributed data were summarised as a mean and standard deviation (SD) for each Group. Nominal categorical data were summarised as a frequency and as a percentage of the total group size.

All subsequent statistical analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA). In all statistical analyses, a P value of <0.05 was considered significant. Baseline demographic and treatment data were compared between control and PHT groups. Continuous data were compared using Mann-Whitney tests. Categorical data were compared using Fisher's exact test.

In statistical analysis of the echocardiographic data the fundamental questions were:

1. For each echocardiographic measure of RV function is there a significant difference between control and PHT Groups?
2. In the PHT Group, is PAP linearly related to RV function (assessed by each echocardiographic measure)?

To answer the first question, RV function data (for each echocardiographic measure) were compared between control and PHT Groups. These data were displayed graphically as a mean and standard deviation of each of the two Groups, for each

echocardiographic measure of RV function. Mann-Whitney tests were used to investigate the statistical significance of differences between the two groups. Mann-Whitney tests were chosen because this test allows comparison of unpaired data sets in which the difference between medians may not be normally distributed.

To answer the second question, linear regression analysis was performed to investigate the relationship between PAP and each measure of RV function. These relationships were presented graphically by plotting each paired measure of RV function (y axis) and PAP (x axis), and generating a "best-fit" line. Linear regression analysis was summarised by reporting the slope and r^2 for each echocardiographic measure of RV function.

2.12 Summary

This chapter has sought to explain how the research study was developed and designed to meet the clinical aim: to improve assessment and treatment of RV dysfunction in infants with PHT. Three fundamental research questions and corresponding hypotheses have been presented and their relevance argued. The development of a case control study design in a neonatal population to test the hypotheses, was discussed. The inclusion, and exclusion, of echocardiographic measures to assess RV function in the study population, the practicalities of obtaining data using these, and analysis of these data have then been explained.

The subsequent chapters present the results of the studies performed according to these methods. The study population characteristics are presented first. Thereafter

each chapter presents RV function data obtained using each specific echocardiographic measure, in both PHT and control groups. A final results chapter presents the paired PAP and RV function data for investigation of the relationship between PAP and RV function.

CHAPTER 3

DESCRIPTION OF STUDY POPULATION

3.1 Introduction

The purpose of this chapter is to describe the study population, and compare baseline characteristics between control and PHT groups. Enrolment to each group is discussed in relation to the entire inpatient population of the NNU at RCH, for the duration of the study period. The characteristics of the subjects in each group are then reported; demographic details, diagnoses, and current treatments. Differences in baseline characteristics between the control and PHT groups, and their significance to inter-group analyses, are discussed.

3.2 Study population enrolment

The study population was enrolled between 12th May 2006 and 30th April 2007. During this period a total of 611 infants were admitted to the NNU at RCH, of whom 38 had a diagnosis of pulmonary hypertension. Nine of these infants had congenital heart disease and were not eligible for enrolment in the PHT group. A further 13 infants were not enrolled for one or more of the following reasons:

- Pulmonary artery pressure of less than two-thirds systemic arterial pressure
- No tricuspid regurgitation jet or no patent arterial duct by which pulmonary artery pressure could be assessed echocardiographically
- The infant was too unstable to tolerate echocardiography
- Access was not available to the patient due to ongoing clinical care
- Access was not available to echocardiography machine
- The study researcher was not available to perform the study

A total of 16 infants were therefore recruited to the PHT group. Twenty-eight infants were recruited to the control group. A further two eligible infants were not enroled to the control group because their parents declined to provide consent for study. Control group size was limited by the duration of the study, access to echocardiography equipment and availability of the study researcher.

3.3 Demographic characteristics

The demographic data are summarised in Table 3.1 for each group, and discussed below.

Table 3.1: Demographic characteristics of control and PHT groups

	Control Group n=28	PHT Group n=16	p
Gestation (weeks)	37.0 ± 3.5	38.5 ± 1.9	0.07
Age (days)	25 ± 20	17 ± 28	0.05
Corrected gestational age (weeks)	40.5 ± 2.4	40.8±3.6	0.82
Sex (male)	20	8	-
Weight (kg)	3.2 ± 0.7	3.3 ± 0.7	0.57

3.3.1 Gestation and age

Mean gestation for control and PHT infants was approximately 38 weeks, and not significantly different between the two groups. Eight infants in the control group were premature with gestational ages at birth from 28 to 36 weeks. Three infants in the PHT group were preterm with gestational ages of 35 to 36 weeks. This is consistent with previous reports that PHT is predominantly, though not exclusively, a disease of the term infant [4].

Corrected gestational age was not significantly different between PHT and control Groups (approximately 40 weeks). Age, in days, ranged from seven to 58 in the control group, and from one to 91 days in the PHT group. The higher mean age in the control group, compared to the PHT group, approached statistical significance (P=0.05). The older age of the control infants reflects the fact that most were recruited and studied when they were stable, after an initial diagnosis had been made and treatment instituted. Very often control infants were studied just prior to their discharge from the NNU. In contrast PHT group infants were generally recruited and studied early in their admission, immediately after a diagnosis of PHT had been made. Eight infants in the PHT group were recruited and studied on day one of life which is consistent with previous reports that most causes of PHT in the newborn present in the first 72 hours of life [112]. Four exceptions were infants who were over two weeks old when they were recruited to the PHT group. Two of these infants were already inpatient in the NNU when the study commenced. The other two infants were referred to the NNU at RCH, from other hospitals, at the ages of six and 12 weeks and subsequently enrolled in the study.

The differences in age of the infants in the two groups is of relevance because RV function is known to change in early post-natal life [67, 113]. With the transition from fetal to ex-utero circulation the RV becomes less thick-walled and more compliant. Accordingly, early diastolic filling is reduced in the fetus and early in post-natal life but increases thereafter. The difference in ages between the control and PHT groups, and its potential influence on RV function, is of relevance when comparing the two groups in subsequent chapters.

3.3.2 Diagnoses

Table 3.2 lists the primary diagnoses of the infants in the PHT group and the degree of pulmonary hypertension demonstrated on initial echocardiogram. In all of these infants PHT was either the primary diagnosis (i.e. idiopathic PHT) or associated with the primary diagnosis (CDH, meconium aspiration syndrome, alveolar capillary dysplasia, vein of Galen malformation).

Table 3.2: Primary diagnoses in the PHT group

Subject	Diagnosis	Pulmonary artery pressure
1	CDH	systemic
2	CDH	supra-systemic
3	CDH	systemic
4	CDH	>2/3 systemic
5	CDH	systemic
6	CDH	systemic*
7	CDH	supra-systemic*
8	CDH	systemic
9	CDH	systemic
10	CDH	systemic*
11	CDH	supra-systemic
12	Idiopathic PHT	systemic
13	Idiopathic PHT	systemic
14	Vein of Galen Malformation	supra-systemic
15	Meconium aspiration	supra-systemic
16	Alveolar-capillary dysplasia	systemic

*PAP assessed from ductal shunt alone

Eleven infants in the pulmonary hypertension group had CDH. Pulmonary hypertension, due to structurally and functionally abnormal pulmonary vasculature, is recognised as a significant cause of morbidity and mortality in CDH, both pre- and post- surgical repair of the diaphragm [114].

Two infants had idiopathic, or primary, PHT. One of these infants also had trisomy 21. No other infants in the PHT group had chromosomal abnormalities. The association between trisomy 21 and idiopathic pulmonary hypertension in the

neonatal period has been previously described although the precise mechanism of elevated PVR is not well understood [115]. The second infant with idiopathic PHT had additional problems of macrosomia, organomegaly and structural brain abnormalities, but no unifying diagnosis.

The primary diagnoses in the remaining infants were meconium aspiration syndrome (MAS), alveolar capillary dysplasia (ACD), and vein of Galen malformation (VGAM). MAS is a relatively common cause of pulmonary hypertension in which failure of the normal fall in PVR at birth is attributed to ongoing hypoxia and activation of the inflammatory cascade. This condition represents a major cause of PHT in the early neonatal period, and carries a significant mortality [112, 116]. Alveolar capillary dysplasia is a rare cause of severe PHT in the newborn, characterised by progressive and severe PHT within the first weeks of life. The condition is characterised by developmental abnormalities and deficiency of the pulmonary microvasculature, and is universally fatal in infancy [117]. VGAM is a rare congenital arterio-venous fistula in the brain. In severe cases VGAM can present with high output cardiac failure and severe pulmonary hypertension in the newborn infant [16]. It has been proposed that PHT in VGAM is due to high blood flows through an otherwise normal pulmonary vascular tree.

The variety of diagnoses in the PHT group reflects the many different causes of PHT in the newborn infant [2]. However, the frequency of these diagnoses in the PHT group may not be representative of their frequency in the entire newborn population. In a study by Walsh-Sukys et al of 385 infants with PHT, admitted to 12 neonatal

units in North America, the commonest causes of PHT were: MAS (41%), idiopathic PHT (17%), pneumonia and/or respiratory distress syndrome (14%), CDH (10%) and pulmonary hypoplasia (4%) [112]. In comparison, the disproportionately high number of infants with CDH in the PHT group reflects the unique nature of the inpatient population at the NNU at RCH. This is the surgical referral centre for all newborn infants with CDH within the state of Victoria, and in the past decade has treated up to 17 CDH infants each year. This experience in managing CDH has led to the development of a treatment protocol (based on "gentle" ventilation, use of inhaled nitric oxide, and prostaglandin to maintain ductal patency) which is associated with significantly improved survival [118]. The assessment of RV function in infants with PHT due to CDH was therefore of particular interest within our institution.

Although the causes of PHT in the study sample, and their relative frequencies, may differ from a wider population, the principle issue in these studies was assessment of RV function in infants with PHT. Even if the causes and mechanisms of increased pulmonary artery pressure may not be representative, the net result on the RV is arguably the same - increased afterload.

None of the control infants had a diagnosis associated with pulmonary hypertension or evidence of pulmonary hypertension on echocardiogram. Thirteen infants had gastro-intestinal disease requiring surgery, two had genito-urinary abnormalities requiring surgery (post-op), five were inpatients for treatment of neonatal infection,

two had gastro-oesophageal reflux and the remaining six had other medical neonatal problems.

3.4 Pulmonary artery pressures in PHT group

In the PHT group, thirteen infants had a tricuspid regurgitation jet from which PAP could be measured. In the remaining three infants, PAP was assessed from ductal shunting patterns. PAPs in each subject are listed in Table 2. One infant had PAP greater than two-thirds peak systemic arterial pressure, but less than peak systemic arterial pressure. Ten infants had PAP approximately equal to peak systemic arterial pressure, and five infants had supra-systemic PAP.

Repeated echocardiograms were performed in the PHT group to investigate the relationship between PAP and RV function. A total of 67 studies were performed (including the 16 initial echocardiograms in each PHT subject). A minimum of two studies and a maximum of eight studies were performed in the same infant. In these 67 studies pulmonary artery pressure could only be quantified (using an acceptable tricuspid regurgitation jet) in 46 studies. These 46 studies were used for later analysis of the relationship between RV function and PA pressure.

3.5 Therapies

The current therapies of each infant were recorded at the time of the initial echocardiogram. All of the infants in the control group were self-ventilating in air; none received mechanical ventilation, inotropic medication, inhaled nitric oxide, sildenafil, prostaglandin E1 infusion or muscle relaxant.

3.5.1 Ventilation

In the PHT group all but one infant was receiving mechanical ventilation. Of those who were mechanically ventilated, eight (50%) received conventional ventilation, four received high frequency oscillatory ventilation (HFOV), and three received high frequency jet ventilation (HFJV). Three of the infants receiving HFOV, and all of the infants receiving HFJV, had CDH. The fourth infant receiving HFOV had severe MAS. HFOV has been found to be effective in MAS and is increasingly used in this setting [119]. HFJV is generally reserved for infants whose lungs demonstrate severe gas trapping or are prone to overdistension, both of which are problems frequently encountered in the ventilation of infants with hypoplastic lungs, as in CDH.

3.5.2 Inhaled nitric oxide (iNO)

Seventy-five percent of infants in the PHT group were receiving inhaled nitric oxide (iNO) at the time of the initial echocardiogram. Dose varied from 7 to 30 parts per million (ppm). iNO, a pulmonary vasodilator, is a mainstay of management of PHT and whose efficacy has been widely demonstrated in the neonatal setting [2, 11].

3.5.3 Sildenafil

Oral sildenafil, a phosphodiesterase type five inhibitor, was being administered to three infants in the PHT group (dose range 1.2 to 1.5 mg/kg per dose, four times per day). These were the oldest infants in the group. Oral sildenafil is increasingly used in infants with severe PHT to facilitate weaning of iNO. However to date there have been no large-scale controlled studies of this agent's efficacy, safety or longer-term outcomes [120, 121].

3.5.4 Inotropes

Intravenous inotropes were being administered to 50% of infants in the PHT group. Five infants received dopamine (5-15 mcg/kg/min), four infants received dobutamine (10-20 mcg/kg/min) and three infants received milrinone, a phosphodiesterase inhibitor (0.25-0.50 mcg/kg/min). Two infants were receiving two inotropes and one infant was receiving all three inotropes. Indications for inotropes were hypotension and/or ventricular dysfunction.

3.5.5 Prostaglandin E_1

Prostaglandin E_1 (PGE_1) infusion was being administered to eight infants (50%) in the PHT group. This agent is employed to maintain ductal patency with the intention of promoting right-to-left shunting through the ductus, thereby providing a "blow-off" valve to a failing right ventricle and reducing RV afterload. PGE_1 may also be a pulmonary vasodilator. Success using PGE_1 in severe PHT has been reported in a number of case series [16, 38, 121]. However, this therapy has not been assessed in

a controlled study, nor have the effects of PGE_1 on RV function been directly assessed.

3.5.6 Muscle relaxant

Thirty-eight percent of infants in the PHT group were receiving muscle relaxant (either intravenous pancuronium or vecuronium). Muscle relaxation is generally reserved for those infants in whom management of pulmonary hypertension and/or respiratory disease is most difficult. Muscle relaxants are proposed to assist ventilation by inhibition of spontaneous breathing and possibly by reducing pulmonary artery pressure. Despite their routine clinical use, there is very limited published evidence supporting the use of muscle relaxation in infants [122].

Is the use of these therapies in the sample group representative of the general population of infants with PHT? In Walsh-Sukys et al's survey of neonatal PHT management in the early 1990's ("pre-iNO" era) 39% of infants received high frequency ventilation (HFOV or HFJV), 84% received inotropic medication, and 73% received muscle relaxant [112]. Compared to these data, the infants in the study group received less inotrope, less muscle relaxant and, understandably, more iNO. This may reflect a widespread change in management practice of PHT in the subsequent decade.

These therapeutic data are also informative in demonstrating the severity of illness in the subjects. The widespread requirement for cardio-respiratory support in the PHT group indicates that this was a group of very sick infants. The control group, in

contrast, required no such therapies, and was a group of stable, relatively well infants.

The difference in therapies between the groups has implications for future comparisons between these groups. None of the infants in the control group were receiving any of the documented therapies whilst 94% of the infants in the PHT group were receiving at least one therapy. Each of the therapies, has the theoretical potential to alter RV function either directly (particularly in the case of inotropic medications) or indirectly by altering preload or afterload. Any comparison of RV function between PHT and control groups must take this into consideration. Any observed difference in RV function between the PHT and control groups could in part be influenced by these therapies, and cannot be attributed to the effects of elevated PAP alone. An ideal control group would be one in which the subjects were receiving the same, or similar, therapies as the PHT group. However, within the time confines of this clinical study, it was not possible to recruit matched control and PHT infants.

3.5.7 Ductal patency

No infant in the control group had a patent arterial duct. In the PHT group fourteen infants had patent arterial ducts (88%), of whom eight (50%) were receiving PGE_1. Average duct diameter was 3.2 mm (range 1.7 to 4.0 mm). As discussed above, the patent ductus may have a theoretical effect on RV function, independent of PAP, by acting as a "blow-off valve" to reduce RV afterload. The difference in ductal

patency between PHT and control group therefore represents another potential reason for differential RV function between these groups.

3.6 Limitations of the study population

The control group of infants were all inpatients in the neonatal unit. They had medical and surgical diagnoses and were, strictly speaking, not "normal", healthy infants. However, these control infants had no evidence of pulmonary hypertension or other cardiovascular disease, and were not receiving any therapy known to alter pulmonary artery pressure or myocardial function. For the purposes of assessing myocardial function they were therefore considered to represent normal controls.

Infants in the PHT group had a variety of diagnoses. Furthermore, the presumed mechanisms of PHT differed within these infants e.g. abnormal pulmonary vasculature in ACD and CDH versus increased pulmonary blood flow in VGAM. It was not possible within the confines of the study duration and setting to recruit a PHT group of infants with a single, consistent diagnosis. However, the majority of infants in the PHT group had CDH. The management of infants with this condition is of particular interest within the unit therefore subgroup analysis of RV function in the CDH subgroup was performed and is presented in the appendix.

As discussed above it was not possible to control for therapies between the PHT and control groups. Consequently, any observed difference in RV function in infants

with PHT, compared to controls, may not solely be due to increased PAP, but other factors, including therapies in this group. Unravelling the individual influences of these factors on RV function is impracticable in a clinical study and beyond the scope of this work. Despite these limitations, it was felt that detecting changes in RV function in infants with PHT in the clinical setting, whichever the causes of these, was still a valid aim with clinical relevance.

3.7 Summary

These data describe the nature of the study population. The control and PHT groups were both composed of term infants of similar gestation, corrected gestational age, and weight. However, whilst the control infants were relatively well, stable infants, the PHT subjects were sick infants receiving varied cardio-respiratory therapy. Differences in age, therapy, and ductal patency between the two groups may each potentially contribute to differences in RV function, independent of the effects of elevated PAP. This hypothesis requires consideration in the analysis of later results. However, it is stressed that the purpose of these experiments was assessment of RV function in infants with PHT and not determination of the sole effects of elevated PAP on RV function. Accordingly, these differences in baseline characteristics do not invalidate further comparison between groups in subsequent chapters.

CHAPTER 4

TRICUSPID VALVE DOPPLER INFLOW VELOCITIES

4.1 Introduction

In this chapter data relating to tricuspid valve Doppler velocities are presented. For each group, data collection and analysis are reported together with summary statistics of E and A wave velocities. Inter-group comparison statistics are then reported and group data plotted to provide an impression of differences between the groups. Finally, in the discussion section the feasibility of the technique is discussed, together with the significance of any inter-group differences in relation to RV function in PHT, limitations of the experiment and of TV Doppler as a technique for assessment of RV function.

4.2 Control Group data

Tricuspid valve Doppler data were obtained in 28 infants in the control group (100%). There were no clinically significant deteriorations during the collection of this data. In two infants in the control group E and A waves appeared to be fused, and the peak velocities of the two waves could not be distinguished. These two infants were therefore not included in further analysis. Fusion of E and A waves is a recognised phenomenon at higher rates [55].

Mean E wave velocity in the control group was 0.52 (0.13) cm/sec. Mean A wave velocities in the group were 0.55 (0.10) cm/sec. Velocities passed a test of normality (Kolmogorov-Smirnov test), indicating Gaussian distribution.

4.3 PHT Group data

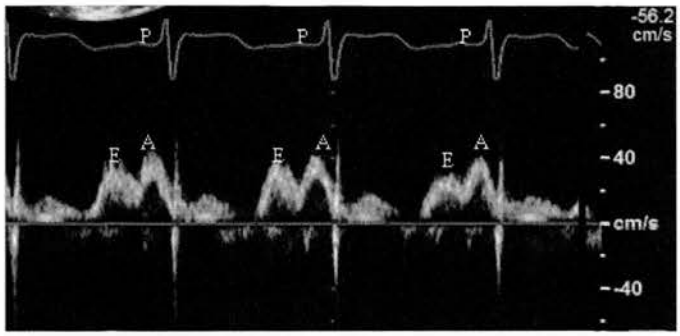
Tricuspid valve Doppler data were obtained in all 16 infants (100%) in the PHT group. There were no clinically significant deteriorations during data collection.

In ten infants in the PHT group no E wave could be identified i.e. no tricuspid valve flow occurred before the P wave on the ECG. In these cases there was an isolated A wave, identified by its commencement after the P wave of the accompanying ECG. An example of such absence of the E wave in a PHT infant is provided in Figure 4.1 below. The absence of a distinct E wave was not thought to be due to E-A fusion for two reasons: firstly, there was no diastolic Doppler wave prior to the P wave on the ECG (i.e. where an E wave would normally occur); secondly, the absence of an E wave occurred in infants with heart rates as low as 130 beats per minute, and not only at higher rates where EA fusion may be predicted.

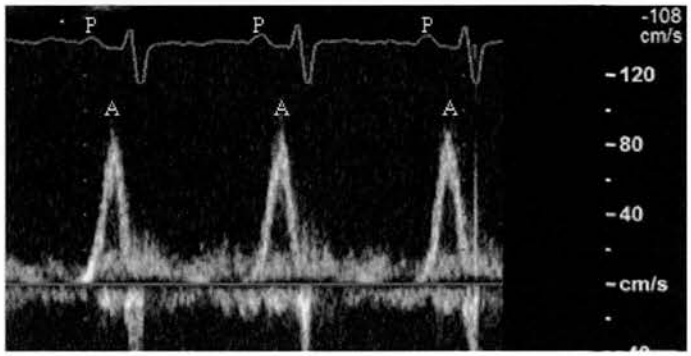
In those ten infants in whom no E wave could be identified, no E wave velocity was recorded. Consideration was given as to whether an E velocity of zero cm/sec should be recorded in these infants and included in statistical analysis. However, this probably invalidates numerical analysis, and therefore numerical group data includes only those infants in whom an E wave could be identified and measured. In these remaining six infants, in whom E waves were present, mean velocity was 0.39 (0.11) cm/sec. A waves were present in all infants in the PHT group with mean velocities of 0.70 (0.22) cm/sec.

Figure 4.1: Tricuspid valve Doppler waveforms; normal and PHT infant

Normal tricuspid valve Doppler waveform; E and A waves clearly present



PHT tricuspid valve Doppler waveform. No E wave is present. An A wave is present occurring after the P wave on the ECG.



4.4 Between group comparison

The absence of E waves in ten infants in the PHT group was the most striking difference between PHT and control groups. In those six infants in the PHT group in whom E waves were present, E velocities were lower than in the control group, although this did not reach statistical significance (P=0.06). Conversely A wave velocity was significantly increased in the PHT group compared to controls.

Summary statistics for comparison between the PHT and control group are provided in Table 4.1. These inter-group differences are represented graphically in Figure 4.2

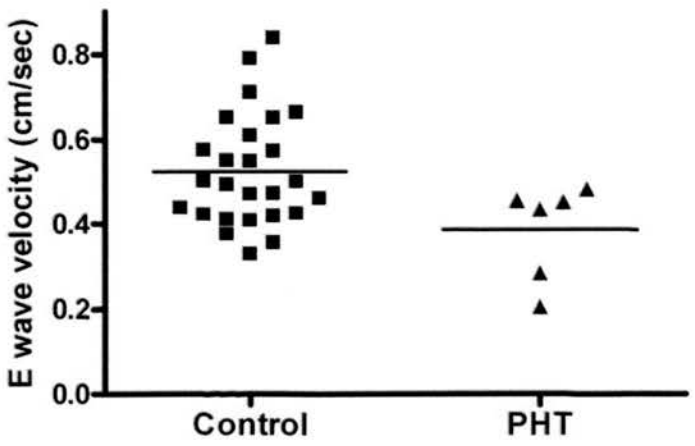
(E wave velocities) and Figure 4.3 (A wave velocities). It is important to note that these summary statistics do not include the E' velocities from those ten PHT infants in whom no E wave was present.

Table 4.1: Peak E and A wave velocities in the control and PHT groups

	Control Group	PHT Group	P
E wave velocity (cm/sec)	0.52 ± 0.13	0.39 ± 0.20*	**0.06**
A wave velocity (cm/sec)	0.55 ± 0.11	0.69 ± 0.22	**0.04**

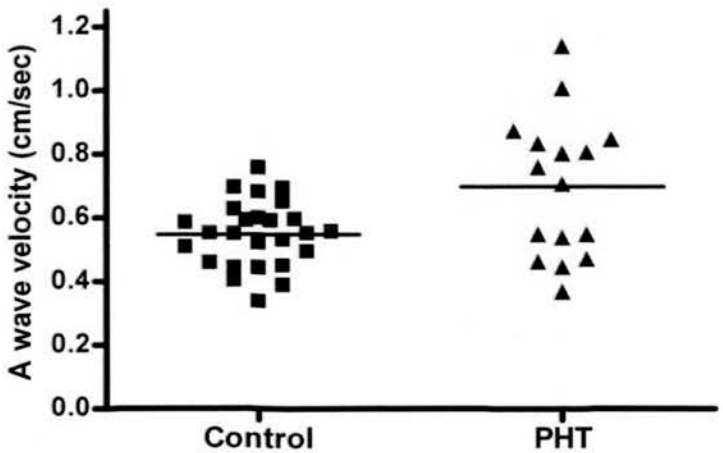
* data from six infants in PHT group in whom E wave identified. No E wave present in remaining 10 PHT infants

Figure 4.2: Tricuspid valve Doppler E wave velocities in Control and PHT infants



Line represents mean. PHT data include only those infants in whom E waves could be identified (n=6). Data from remaining ten PHT infants, in whom no E wave identified, is not included in this figure.

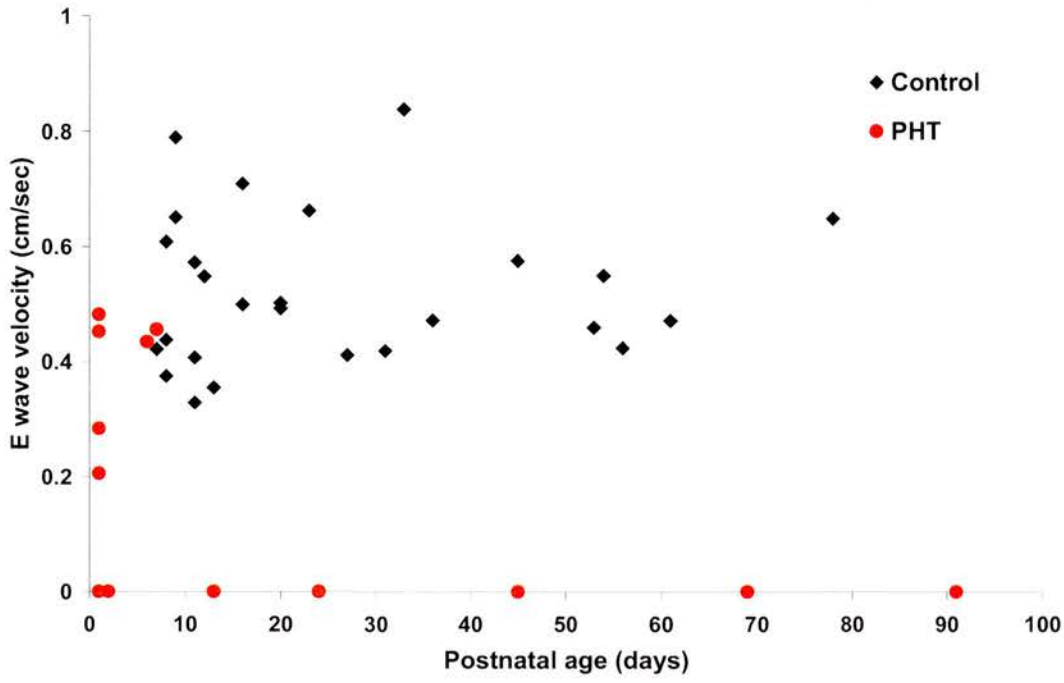
Figure 4.3: Tricuspid valve Doppler A wave velocities in Control and PHT infants



Line represents mean.

The potential for heart rate differences or age differences between the groups to contribute to the observed differences in Doppler velocities was considered. Tricuspid Doppler velocities are dependent on heart rate [55] and therefore the observed between–group differences could potentially have been due to heart rate differences between the control and PHT group. However, in this study, mean heart rates were not significantly different between the two groups (138 (15) vs. 150 (17), P=0.07). Diastolic RV function is also dependent on postnatal age: in the fetus early diastolic filling is reduced and the A wave is dominant [67]. The possibility that differences in postnatal age could contribute to the inter-group difference in E wave velocity was investigated by plotting E wave velocity against age (in days) for each group, as seen in Figure 4.4. For the purpose of this analysis (but not inter-group statistical analysis) those PHT infants in whom no E wave was present were attributed an E velocity of 0 cm/sec. The important point, shown in this figure, is that E wave velocities were lower in the PHT group at all ages, including the youngest infants. This does not support the hypothesis that differences in E wave velocity are due to age differences between the groups

Figure 4.4: E wave velocity and postnatal age



E velocities tended to be lower in the PHT group than the control group, independent of postnatal age. (NB Infants in PHT group in whom no E wave present were attributed a velocity of zero cm/sec.)

4.5 Discussion

4.5.1 Feasibility

Tricuspid valve Doppler is an established technique in infants [55, 113]. The current data confirm the feasibility of tricuspid valve Doppler in normal and PHT infants. This assertion is based on the findings that the technique could be performed safely in 100% of infants in both the control and PHT groups, that meaningful quantitative measures (of E and A wave velocity) could be made, and that intra-group velocities demonstrated an acceptable level of variance.

4.5.2 Reduced E wave velocity: comparison with previous reports and significance

The most striking finding in this experiment was the reduction in, or absence of, E wave velocities in the PHT group. Indeed, in 63% of infants in this group the E wave was entirely absent indicating complete loss of early diastolic filling. These findings are in agreement with previous demonstrations of reductions in E wave velocity in adults with PHT [66], but have not been previously reported in infants with PHT.

A similar pattern of impaired diastolic filling is seen in the left ventricle in some adults with ischaemic heart disease, hypertension, and cardiomyopathy [123-125]. In these patients the same pattern of reduced E wave velocity, described as "*type 1 diastolic dysfunction*", is attributed to an inherent impairment of myocardial relaxation.

The absence of, or reduction in, E wave velocities in this study might also be considered to indicate impaired myocardial function (reduced active relaxation and/or reduced passive compliance) in the RV of infants with PHT. However, atrio-ventricular valve Doppler velocities are not solely dependent on myocardial function, but are also highly load-dependent i.e. altered by preload and afterload [26]. The trans-tricuspid velocities are determined by the instantaneous pressure gradient across the valve. This is the difference between right atrial pressure (RAP) and RV diastolic pressure RVEDP. RAP is both preload and afterload dependent. RVEDP is, in turn, determined by myocardial properties (active relaxation and passive

compliance) and by afterload (PAP) [26]. Doppler velocities are therefore highly load-dependent [126].

The observed absence of, or reduction in, E wave velocity could therefore reflect a reduction in RAP or an increase in RVEDP independent of, or as well as, a change in myocardial function. It is conceivable, for example, that use of ventilation or vasodilators in the PHT group could have contributed to a reduced preload and therefore reduced RAP in this group. Alternatively, the increase in PAP, producing an increased afterload in the RV, may be sufficient to produce the observed changes in E wave velocity.

4.5.3 Increased A wave velocity: previous reports and significance

The significant increase in A wave in the PHT group has not been previously reported in infants with PHT. It is hypothesised that this may represent increased atrial contraction in these infants to generate increased late diastolic RAP. This would serve as a compensatory mechanism to maintain RV diastolic filling in the face of reduced early diastolic filling in PHT. Indeed, studies in adults have demonstrated increased RA contractility in chronic pulmonary hypertension [127]. Stretch-induced changes in intracellular calcium may be important in mediating this increase in atrial myocyte contractility [128]. An alternative, but not mutually exclusive explanation is that the apparent increase in A wave size in fact represents abnormally delayed ventricular relaxation occurring late in diastole and coinciding with atrial contraction.

4.5.4 Limitations of this study

Failure to minimise the angle of insonation of the Doppler beam through the transtricuspid flow may have led to under-measurement of peak velocities. However, care was taken, even in those infants with cardiac malpositioning (e.g. in congenital diaphragmatic hernia), to maintain an angle of insonation of less than fifteen degrees. Sub-optimal Doppler image quality and "spectral widening" of the Doppler waveform may have led to error in post-acquisition measurement of Doppler velocities. Furthermore, during data analysis the researcher was not blinded to control and PHT group data, which may have allowed introduction of bias.

4.5.5 Limitations of tricuspid valve Doppler as a measure of RV function

As discussed above, the biggest limitation of TV Doppler as a measure of RV function is the load-dependence of this measure. It is unknown whether changes in TV Doppler velocities, in particular E velocity, are the direct consequence of changes in loading, or actual changes in myocardial function. Consequently TV Doppler, though a rapid and practicable technique, is limited as a measure of RV myocardial function, when used in isolation.

4.6 Conclusion

In this chapter the tricuspid valve Doppler velocities in the control and PHT groups have been reported and compared. This data has confirmed that tricuspid valve Doppler is a feasible technique in newborn infants. In infants with PHT early

diastolic (E wave) velocities were reduced, or absent, although the difference between groups did not reach statistical significance. This may be suggestive of impaired diastolic myocardial RV function. However, different loading conditions in the PHT group may also have contributed to the impaired early diastolic filling, highlighting the load-dependence of tricuspid valve Doppler as a measure of myocardial function. Increases in A wave velocity in the PHT group, meanwhile, suggest increased late diastolic filling due to atrial contraction.

CHAPTER 5

RIGHT VENTRICULAR OUTPUT

5.1 Introduction

In this chapter the data relating to measurement of RVO are presented. The echocardiographic data acquired for RVO calculation are each reported first i.e. pulmonary valve (PV) diameter, velocity time integer (VTI) and heart rate (HR). RVO, calculated using these data, is then reported. Results of inter-group comparison are provided for each of the echocardiographic measures and for RVO. In the discussion section, the experimental data are interpreted in relation to feasibility of the technique, agreement with previously published data, and the significance of any differences between the PHT and control groups. Finally, limitations of the study, and limitations of RVO as a technique are discussed.

5.2 Data collection and analysis for RVO calculation

Echocardiographic data required for calculation of RVO were successfully obtained in all 28 infants in the control group (100%), and all 16 infants in the PHT group (100%), without significant clinical deterioration. All data were successfully analysed to allow measurement of PV diameter, HR, VTI, and subsequent calculation of PV cross sectional area (CSA), stroke volume (SV) and RVO. Results for each of these measures are reported below and summarised in Table 5.1.

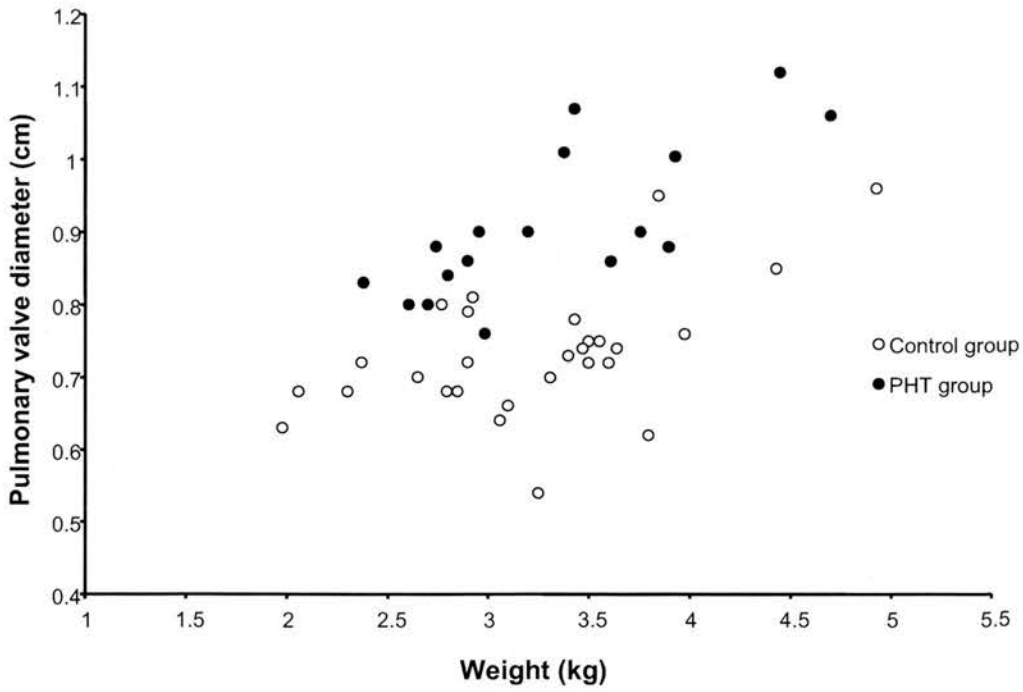
5.3 Pulmonary valve diameter and cross sectional area data

PV diameters for each group are summarised in Table 5.1. Control group PV diameter was 0.73 (0.09) cm (range 0.54-0.96cm). PHT group PV diameter was 0.91 (0.11) cm (range 0.76 to 1.12 cm), and significantly elevated compared to the control

group (P<0.0001). Pulmonary valve cross sectional area in the control group was 0.43 (0.10) cm^2, and in the PHT group 0.60 (0.16) cm^2. The increased valve area in the PHT group was statistically significant (P<0.0001).

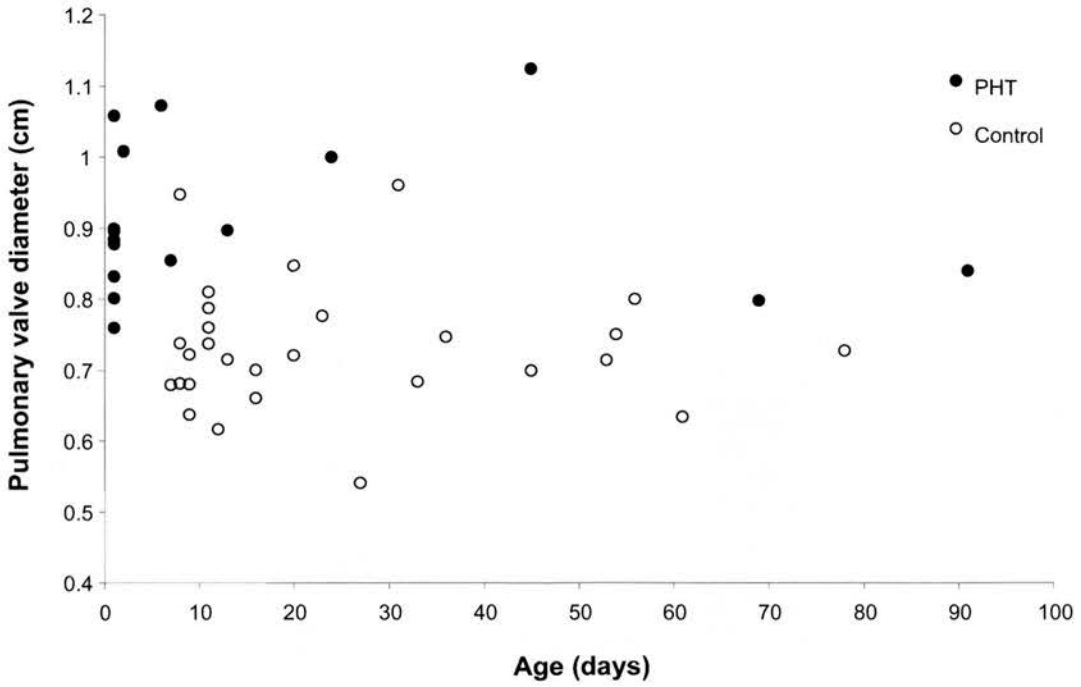
To investigate whether the inter-group difference in valve diameter (and CSA) was related to differences in infant weight, PV diameter was plotted against weight for each infant in both groups (Figure 5.1). It can be seen that, for all weights, PV diameter was lower in the control infants than the PHT infants, suggesting a genuine increase in valve diameter in the PHT group, not related to greater infant size in this group.

Figure 5.1: Pulmonary valve diameter and weight



To investigate whether increased valve diameter in the PHT group was present from birth, or developed postnatally, valve diameter and infant age were plotted for each infant (Figure 5.2). As demonstrated in this figure PV diameter tended to be higher in the PHT group at all postnatal ages, including the first days of life. This may suggest that increased valve diameter in the PHT group is present from before birth.

Figure 5.2: Pulmonary valve diameter and age



5.4 Velocity time integer (VTI)

Pulse wave Doppler waveforms of pulmonary valve flow were analysed offline to obtain velocity-time integers (VTI). Control group mean VTI was 14.4 (1.6) cm. PHT group mean VTI was 10.2 (4.1) cm. This difference was significant (P=0.004).

5.5 Heart rate

HR was obtained from the ECG accompanying pulmonary valve Doppler data. Mean HR in the control group was 138 (18) beats per minute (bpm). In comparison, heart rate in the PHT group was significantly elevated at 154 (19) bpm, P= 0.016.

5.6 RVO

RVO was calculated, as discussed in Chapter 2, using VTI, valve cross-sectional area and heart rate data from each group, inserted into the equation:

$$RVO = \frac{VTI \times heart\ rate \times CSA}{weight}$$

Mean RVO in the control group was 273 ± 81 mL/kg/min (range 134 to 518 mL/kg/min), and in the PHT group was 308 ± 133 mL/kg/min (range 140 – 652 mL/kg/min). This difference was not significant (P=0.60).

The product of VTI and CSA is SV:

$$SV = VTI \times CSA$$

SV represents the volume of blood ejected across the pulmonary valve with each heart beat, and was calculated and reported here as it gave some indication of myocardial function, independent of heart rate. Mean SV in the control group was 6.1 (1.7) mL/kg, and in the PHT group 7.0 (4.0) mL/kg. This difference was not statistically significant (P=0.91).

Table 5.1: Echocardiographic parameters related to RVO calculation

	Control group mean (SD)	**PHT group** mean (SD)	**P**
Pulmonary valve diameter (cm)	0.73 (0.09)	0.91 (0.11)	<0.0001
Pulmonary valve cross-sectional area (cm^2)	0.43 (0.10)	0.60 (0.16)	<0.0001
Velocity time integer (cm)	14.4 (1.6)	10.2 (4.1)	0.004
Heart rate (bpm)	138 (18)	154 (19)	0.016
Stroke volume (mLs/kg)	6.1 (1.7)	7.0 (4.0)	0.91
Right ventricular output (mL/kg/min)	273 (81)	308 (133)	0.60

5.7 Discussion

5.7.1 Feasibility

RVO measurement in infants is a well established technique, the feasibility of which was confirmed in control and PHT infants in this study. [65].

5.7.2 Agreement between RVO in controls and existing normative data

Mean cardiac output in newborn infants, measured using indicator dilution techniques and Doppler echocardiography of left ventricular output, has previously been reported as being in the range 246 mL/kg/min and 265 mL/kg/min [129-131]. Normative RVO data, obtained using pulse wave Doppler echocardiography, has been reported once before in a population of normal term and preterm infants, by Tsai-Goodman et al [65]. Mean RVO in that study was 255 mL/kg/min. The slightly higher mean RVO in our control population may be due to a genuine increase in cardiac output in this group. Five infants in the PHT group had diagnoses which might have led to increased cardiac output, i.e. infection and metabolic disease. Another explanation for higher RVO in our control group is measurement error, which is a particular limitation of RVO measurement, discussed later in this section.

5.7.3 Inter-group differences in RVO

There was no statistically significant difference between RVO in the PHT group and control groups. There was, however, a non-significant trend toward higher RVO in the PHT group which, if genuine, may be secondary to significant right-to-left shunting via a PDA, which was present in 14 infants (88%). In these infants, RVO represents a combination of pulmonary blood flow *and* a proportion of systemic blood flow, and should therefore exceed the equivalent RVO in a normal infant without shunts (e.g. the control infants in this study). Indeed, RVO up to twice

normal values has previously been reported in infants with right-to-left ductal shunts in PHT due to VGAM [16, 132].

Another explanation for high RVO in the PHT group is increased metabolic rate in these sick infants. The infants in the PHT group tended to be sicker with diagnoses associated with increased metabolic demand.

5.7.4 Inter-group differences in heart rate and stroke volume

Heart rate was significantly increased in the PHT group, whilst SV was not significantly different. This suggests that RVO in PHT infants is maintained by chronotropic (heart rate-dependent mechanisms) rather than by an increase in myocardial function. It may be hypothesised that myocardial function, in the PHT group, was depressed or could not be increased further, hence the requirement for an increase in HR. Analysis of RVO alone, however, can give no indication of the relative contributions of heart rate, myocardial function and loading conditions. This highlights again, the limitation of RVO as a "global" measure of RV function, which cannot be used to measure myocardial function in isolation.

5.7.5 Intergroup differences in pulmonary valve diameter

It was an unexpected finding that valve diameter was significantly higher in the PHT group compared to controls. VTI was, conversely, lower in the PHT group, indicative of lower trans-valvar flow velocities across a wider valve. The overall effect, as discussed above, was that RVO did not differ between the two groups. The increased valve diameter in the PHT group was seen in infants of all weights.

The reasons for an increase in PV diameter in infants with PHT are unclear. Increased afterload, and increased flow (RVO), may conceivably lead to distension, or "stretch" of the valve in PHT. However, we observed the increased valve diameter in infants with PHT at all ages, including within the first day of life. This suggests that increased PV diameter is a developmental phenomenon in fetal life. Since high PAP, and high RVO, are universal findings in the normal fetus, then some another developmental factor in the PHT infants, may be responsible for increased PV diameter. This important, co-incidental finding represents an interesting area for further study. Of note, Suda et al have previously observed that PV diameter tended to be *smaller*, compared to normal, in infants with CDH [133]. However, their study involved retrospective analysis of echocardiographic data, which may not have included optimal views for measuring valve diameter. Further investigation, with larger study populations may help to identify whether PV diameter is genuinely different in PHT.

5.7.6 Limitations of this study

The researcher was not masked to the diagnosis during data analysis, with the potential that bias may have been introduced. The echocardiographic images were also of variable quality. Image quality tended to be worst in those infants with lung hyperinflation and/or significant parenchymal lung disease, in whom ultrasound "windows" were small and image resolution poor. Error may have been introduced in the measurement of valve diameter from poorer quality two-dimensional images, in which identification of valvar hinge points was difficult. Repeated measurement

of valve diameter, over five consecutive cardiac cycles in each infant, was performed in an attempt to reduce this measurement error. Similarly, Doppler image quality may have affected VTI measurements. In poorer quality images spectral broadening of the Doppler waveform may have led to overestimation of VTI.

5.7.7 Limitations of RVO as a measure of myocardial function

As discussed above, a major limitation of RVO is that it is a global measure determined by preload, myocardial function, heart rate and afterload. The individual contribution of myocardial function to RVO cannot be distinguished.

A second limitation of any Doppler measure of cardiac output, including RVO, is the potential for high levels of variability (error) in measurement [103]. Total variability, in RVO measurement, is a combination of physiological variability and intra-observer variability (repeatability) or inter-observer variability (reproducibility). Measurement variability (measurement error) may be introduced in the collection of each of the measures required for the calculation of RVO, particularly measurement of pulmonary valve diameter [65]. Measured valve diameter may vary depending on 1) whether the valve has been imaged across its maximum diameter, 2) the time in the cardiac cycle at which the diameter is measured, 3) the position within the right ventricular outflow tract at which valve diameter was measured and 4) the quality of the echocardiographic images (as discussed above). Any error in valve diameter is further increased by squaring this measure to obtain CSA. It was not possible to measure repeatability of RVO measurements in this study. Previous studies have reported co-efficients of variation,

for both intra-observer variability and inter-observer variability, of Doppler cardiac output as high as 22% [103].

The calculation of RVO is also a time consuming process. Although the time taken to derive RVO was not formally measured in this study, it was the researcher's experience that the entire process, including obtaining all data, performing analysis and calculation, took at least 30 minutes per infant. This included repeating measures over five cardiac cycles.

5.8 Conclusion

The data presented in this chapter has confirmed the feasibility of measuring RVO in normal infants and infants with PHT. RVO was not significantly different in the PHT group compared to the control group. However this study has also highlighted limitations of RVO as a measure of myocardial function. The "global" nature of RVO, with its load and heart-rate dependence, means that it cannot be used specifically to assess RV myocardial function. Indeed, the observation that HR was increased in the PHT group, whilst SV did not differ, suggested that myocardial function was not increased (or may even have been be decreased) in the PHT group, despite no difference in RVO. Furthermore, the presence of significant ductal shunts in the PHT group indicates the confounding influence that shunts may have on RVO, invalidating comparison with "normal" RVO values. The potential for error to be introduced at various stages in the calculation of RVO brings into question the repeatability of this technique. The time consuming nature of RVO calculation also makes it impracticable for use in the clinical setting.

The finding of increased PV diameter, independent of age and weight, in the PHT group was unexpected and merits further investigation.

CHAPTER 6

RIGHT VENTRICULAR

MYOCARDIAL PERFORMANCE INDEX

6.1 Introduction

In this chapter, the data relating to measurement of the RV myocardial performance index (RV_{MPI}) are presented. Firstly, the experience of obtaining and analyzing tricuspid valve and pulmonary valve Doppler data is reported. Next, the Doppler time intervals, measured for RV_{MPI} calculation are reported, as is heart rate for each group. The calculated RV_{MPI} is then reported together with inter-group comparison statistics. The relationship between RV_{MPI} and age is also reported for each group to investigate whether age differences between the groups might contribute to differences in RV_{MPI}. In the discussion section the feasibility of RV_{MPI} is considered. Any differences in RV_{MPI} between PHT and control groups are discussed in relation to RV myocardial function. Comparison is made with prior reports of RV_{MPI} in PHT states. Limitations of this experiment, and of RV_{MPI} as a technique for assessing RV function, are then discussed.

6.2 Data collection and analysis

Tricuspid and pulmonary valve Doppler data for RV_{MPI} calculation were obtained in 28 infants (100%) in the control group and 16 infants (100%) in the PHT group. All echocardiograms were well tolerated without clinically significant deterioration.

Tricuspid valve Dopplers used for RV_{MPI} calculation were the same as those collected for measurement of diastolic trans-tricuspid velocities in Chapter 4.

Pulmonary valve Dopplers obtained for RV_{MPI} calculation were the same as those obtained for RVO calculation in Chapter 5.

In five infants in the control group echocardiographic data could not be analysed to obtain Doppler time intervals. This appeared to be a problem with the analysis software on the Phillips IE33 which prevented measurement of time intervals from echocardiographic data which had been stored on DVD and re-uploaded to the IE33. This problem could not be resolved despite assistance from the manufacturer. The same problem was not encountered in analysis of the remaining 23 control studies directly from the inbuilt hard-drive of the IE33. All 16 studies in the PHT group were successfully analysed.

6.3 Doppler derived time intervals for RV_{MPI} calculation

Doppler time intervals were measured for RV_{MPI} calculation as described in Chapter 2 and are summarised, for each group, in Table 6.1 below. The time interval from cessation of ventricular filling in one cardiac cycle to commencement of ventricular filling in the next cycle (a) was measured from the tricuspid valve Doppler. Mean "*a*" duration was 249 (25) milliseconds in the control group and did not differ significantly in the PHT group; 263 (33) msec, P =0.14. Right ventricular ejection time (*b*) was measured from the accompanying pulmonary valve Doppler waveform of each infant. Mean "*b*" duration was 201 (15) msec in the Control Group and significantly shorter in the PHT group; 171 (21) msec, P<0.0001.

Table 6.1: Doppler-derived time intervals in control and PHT groups

	Control Mean (SD)	**PHT** Mean (SD)	**P**
a (milliseconds)	249 (25)	263 (33)	0.14
b (milliseconds)	201 (15)	171 (21)	<0.0001

a, time between cessation of and commencement of tricuspid inflow obtained from tricuspid valve Doppler. ***b***, right ventricular ejection time obtained from pulmonary valve Doppler.

6.4 Heart rate

Mean HR in the control group, at the time of obtaining RV_{MPI} data, was 138.2 (16.3) bpm. Mean HR in the PHT group was significantly elevated compared to controls: 154.3 (17.3) bpm, P=0.01. Comparison of HR between the groups was important, since differences in HR may contribute to differences in RV_{MPI}, independent of changes in myocardial function.

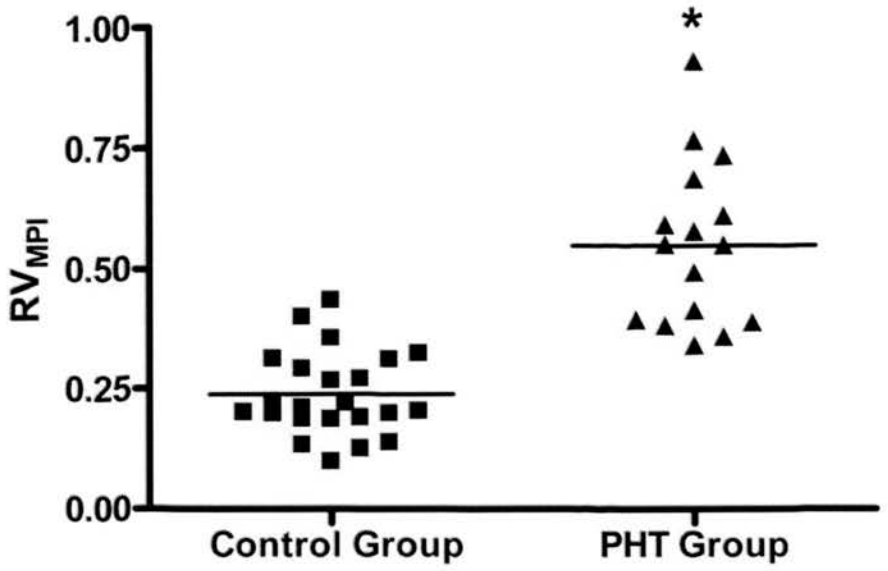
6.5 Calculated RV_{MPI}

RV_{MPI} was calculated as described in Chapter 2, using the following formula:

$$RV_{MPI} = \frac{a-b}{b}$$

RV_{MPI} in the control group was 0.24 (0.09), and 0.55 (0.17) in the PHT group. The increased RV_{MPI} in PHT was statistically significant, P<0.0001. RV_{MPI} in the control and PHT groups is plotted in Figure 6.1 to demonstrate the inter-group difference.

Figure 6.1: RV_{MPI} in control and PHT infants

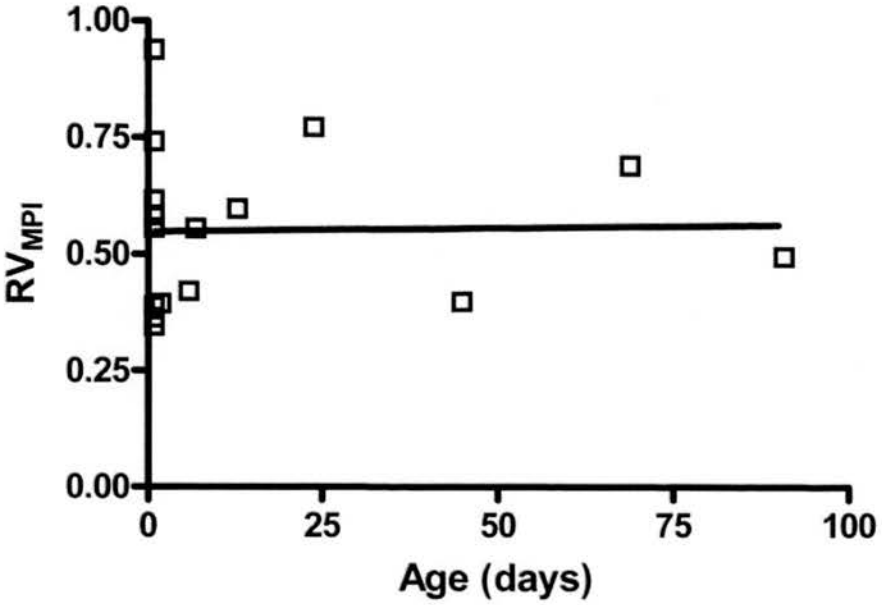


* P<0.0001. Line represents mean for group.

6.6 RV_{MPI} and age

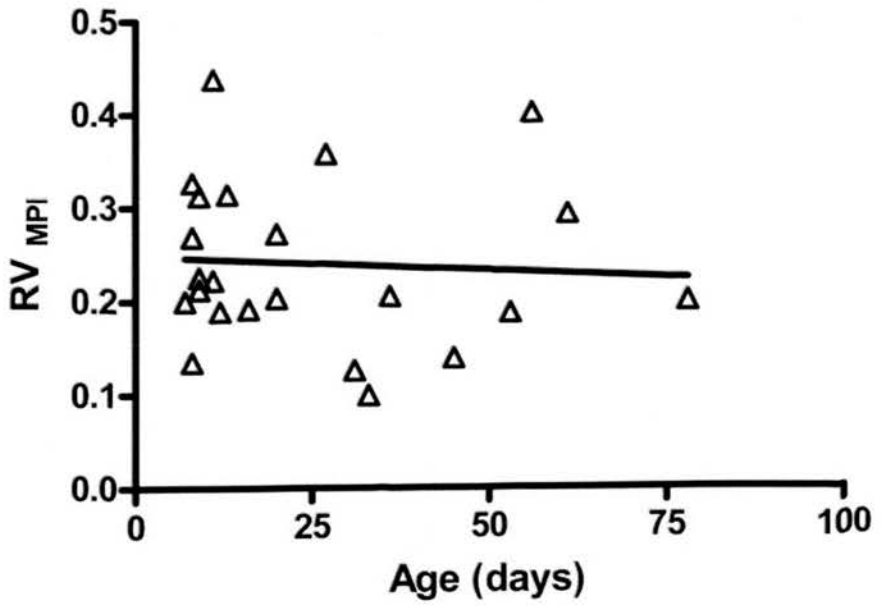
A previous study has observed that RV_{MPI} falls within the first days after birth [72]. The control group were significantly older than the PHT group, in terms of postnatal age (see Chapter 3), raising the possibility that the difference in RV_{MPI} might have been due to age differences between the groups. The relationship between RV_{MPI} and age was therefore investigated for each group using linear regression analysis. RV_{MPI} did not correlate with age in the PHT group (r^2=0.001, P=0.90; Figure 6.2) or in the control group (r^2=0.005, P=0.76; Figure 6.3). That RV_{MPI} did not correlate with age in either group suggests that differences in RV_{MPI} cannot be attributed to age differences between the two groups.

Figure 6.2: Relationship between RV_{MPI} and age in PHT group



r^2=0.001, p=0.90

Figure 6.3: Relationship between RV_{MPI} and age in control group



r^2=0.005, p=0.76

6.7 Discussion

6.7.1 Feasibility

This study has confirmed the feasibility of measuring RV_{MPI} in infants, including, for the first time, those with PHT. The tricuspid and pulmonary valve Dopplers required for RV_{MPI} calculation could be obtained rapidly and were well tolerated. The technical issue which prevented measurement of Doppler time intervals, and hence RV_{MPI} calculation in five control infants, appeared to be a problem peculiar to the Phillips IE33 machine used for these experiments.

6.7.2 RV_{MPI} in the control group: comparison with previous reports

RV_{MPI} in the control group was in good agreement with previous reports in comparable normative study populations (Table 6.2). Malakan-Rad et al measured RV_{MPI} in a group of 51 infants in the first 48 to 72 hours of life and reported a mean RV_{MPI} of 0.23 (0.14). Borzoee et al, studying a population of 108 children, aged 3 days to 18 years reported an RV_{MPI} of 0.25 (0.09) [134]. Similarly, Ishii et al reported a mean RV_{MPI} of 0.24 (0.04) in 150 healthy children aged 30 days to 18 years [111].

Table 6.2: RV_{MPI} in normal infants: comparison of current data with published literature

Study	n	Age	RV_{MPI}
Borzoee et al [134]	108	3 days to 18 years	0.25 (0.09)
Malakan-Rad [135]	51	48 to 72 hours	0.23 (0.14)
Ishii [111]	150	30 days to 18 years	0.24 (0.04)
This study (control group)	23	7 to 78 days	0.24 (0.09)

6.7.3 Elevated RV_{MPI} in PHT: significance and comparison with previous reports

This experiment demonstrated, for the first time, elevated RV_{MPI} in infants with PHT. This is in good agreement with previous reports of elevated RV_{MPI} in older children and adults with PHT. Yeo et al demonstrated a raised RV_{MPI} (median 0.83) in a group of 53 adults with primary pulmonary hypertension [104]. Dyer et al subsequently reported increased RV_{MPI} (0.64±0.30) in children with idiopathic pulmonary hypertension [75]. However, the patho-physiological processes responsible for chronic PHT in these paediatric and adult populations may differ from those in newborn infants. It cannot therefore be assumed that the increase in RV_{MPI} in our control population is produced via the same mechanisms. The only prior report of RV_{MPI} in a "newborn" population, was by Sugiura et al, who have similarly demonstrated an increased RV_{MPI} in a newborn piglet model of hypoxia-induced pulmonary hypertension [73].

What is the mechanism and significance of a raised RV_{MPI} in PHT and can it be considered an indicator of RV myocardial dysfunction? RV_{MPI}, like RVO, is a "global" measure of circulatory function, which is dependent on myocardial function and loading conditions. Vogel et al have demonstrated this load dependence of RV_{MPI} in a pig model in which RV_{MPI} was observed to change in the face of manipulations of preload and afterload, without changes in myocardial function [76]. The elevated RV_{MPI} in the experiments presented here may therefore be an indication of changing load, in particular increased afterload, and not changes in myocardial function *per se*. Even if elevated RV_{MPI} did reflect a change in RV myocardial

function, it provides no information on the mechanism of this change i.e. the relative contributions of systolic and diastolic function. Furthermore, because of the way the index is calculated, a decrease in systolic function could conceivably be masked by an increase in diastolic function and vice versa.

Examination of the individual time intervals used to calculate RV_{MPI}, provides further information on the possible mechanisms of RV dysfunction in the PHT group. RVET was shorter in the PHT group compared to controls, whilst time period "*a*" was not significantly different. *a* is the sum of IVRT, isovolumic contraction time (ICT) and RV ejection time (RVET). It follows that a longer IVRT and/or longer isovolumic contraction time (ICT) must have produced the higher RV_{MPI} in the PHT group. ICT may be expected to increase in the face of increased afterload, as the contracting ventricle must generate increased pressures before the pulmonary valve can open and ventricular ejection can occur. If IVRT were lengthened then this would suggest that some degree of diastolic dysfunction would also be present. However, the experimental data gave no indication of relative changes in ICT or IVRT.

The age of the infants in the two groups required consideration when interpreting the observed difference in RV_{MPI}. Tsutsumi et al have previously reported that RV_{MPI} is age dependent, being higher in fetal life and at birth, but falling acutely within the first 48 hours of life to approximately 0.25 to 0.3 [72]. However, Borzoee found no correlation between age and $RV_{MPI,}$ in infants over 48 hours old [134]. Analysis of the current experimental data also found no correlation between RV_{MPI} and postnatal

age. It was therefore felt that inter-group differences in RV_{MPI} could not be attributed to the age difference between the younger PHT group and older control group.

6.7.4 Limitations of this study

This study was not blinded in data analysis or collection, with the result that observer bias may have been introduced. The difference in heart rates between the PHT and control groups may have directly altered RV_{MPI} and confounded the results. At higher heart rates, diastole is shortened, and RV_{MPI} might be expected to decrease. However in this experiment, the PHT group had a higher mean HR yet RV_{MPI} was still significantly increased in this group. In an ideal experiment, in which HR was the same in PHT and control groups, RV_{MPI} might reasonably be expected to be even higher in the PHT group than that observed in the current study.

Physiological variation in heart rate when obtaining the separate tricuspid and pulmonary valve Doppler measurements, in each infant, may also have confounded RV_{MPI} calculation. Therefore care was taken to obtain these two measures consecutively and over the shortest period of time.

6.7.5 Limitations of RV_{MPI}

A major limitation of RV_{MPI}, as discussed above, is that it is a "global" measure, and gives no indication of relative systolic or diastolic function. In addition the index is highly load-dependent, and in the setting of PHT it cannot be known whether changes in RV_{MPI} reflect genuine changes in myocardial function or simply changes in afterload.

6.8 Conclusions

RV_{MPI} can be easily measured in infants, and is elevated in those with PHT. Although this increase in RV_{MPI} may represent a genuine change in myocardial function in PHT the direct effects of preload and afterload may also have contributed to the observed difference. Furthermore, RV_{MPI} could not distinguish specific changes in systolic and diastolic function. This highlights the global nature and load-dependence of RV_{MPI} which limit its use as a clinical measure of RV myocardial function.

CHAPTER 7

PULSE WAVE TISSUE DOPPLER IMAGING

7.1 Introduction

In this chapter the data relating to use of PWTDI assessment of RV function in normal infants and infants with PHT are reported. The experience of PWTDI data collection and analysis are discussed first. Next, PWTDI systolic velocities, diastolic velocities and diastolic time intervals are presented. Summary statistics are provided for each group together with inter-group comparison statistics. Group data is presented graphically, when relevant, to highlight inter-group differences. The relationship between diastolic velocities and age, and diastolic velocities and heart rate were also considered. In the discussion section the feasibility of PWTDI in the infant RV is considered in light of the study findings. Finally, between-group differences in PWTDI data are discussed in relation to mechanisms of RV dysfunction in PHT.

7.2 Data collection and analysis

PWTDI data were obtained in 25 (89%) infants in the control group and 16 infants (100%) in the PHT group. In the three remaining infants in the control group, PWTDI data could not be obtained because study echocardiograms were discontinued to allow routine clinical care of the infants. All echocardiograms which could be performed to collect PWTDI data were well tolerated without clinically significant deterioration.

Analysis of PWTDI data was performed offline as described in Chapter 2, to obtain systolic and diastolic time velocities and diastolic time intervals. All 25 collected

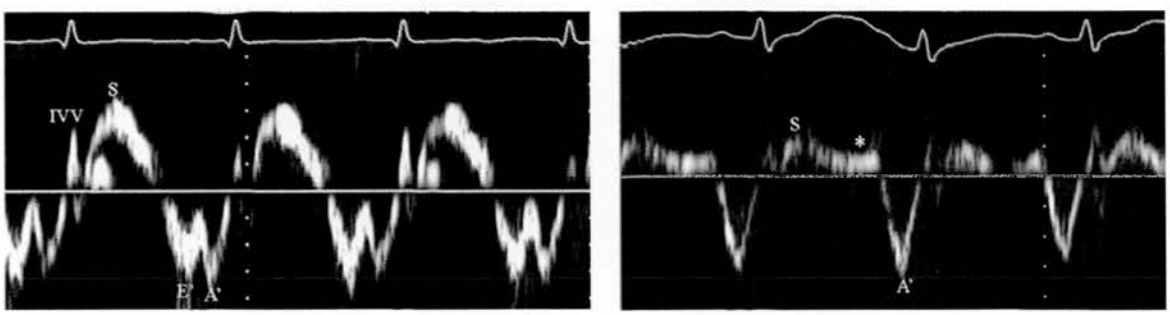
PWTDI studies in the control group were successfully analysed. Fifteen (94%) of the PWTDI studies in the PHT group were also analysed successfully. In the one remaining infant in the PHT group, PWTDI data from the RV could not be analysed due to artefact on the Doppler waveform caused by high frequency oscillatory ventilation (HFOV). This problem was not encountered in analysis of the data from the other three infants who were also receiving HFOV, nor in the three infants receiving HFJV. All subsequent RV PWTDI data presented in this chapter refer to the 15 infants in whom RV data could be analysed.

The systolic velocities IVV and S were measured in all infants. However, it was found that IVA could not be accurately measured from PWTDI data because of spectral broadening of the Doppler waveform. Even at the highest recording speed (150mm/sec), and with gain settings optimised, the upslope of the isovolumic velocity was too steep and the trace too broad to allow meaningful measurement of the slope (IVA).

The diastolic velocities E' and A' were also measured in all infants in whom PWTDI analysis could be performed. In eight infants in the PHT group no distinct E' velocity could be identified, and instead only an isolated single diastolic velocity was present (Figure 7.1). This single velocity was identified as an A' wave because of its occurrence after the P wave of the corresponding ECG. The absence of an E' wave in these infants was considered to be a genuine and significant finding, as is discussed later in this chapter. However, as no E' velocity could be measured, the data from these infants was not included in numerical statistical analysis.

It was intended that the diastolic time period IVRT be measured in the RV and IVS in all infants in whom PWTDI data could be analysed. However, difficulty was encountered when measuring IVRT in the RV in four infants in the PHT group. In these infants the cessation of the S wave and the beginning of the E' wave (i.e. the IVRT period) could not be accurately identified due to the presence of an unexpected positive velocity after and continuous with the S wave (see Figure 7.1 below). This positive velocity was designated a "post-systolic contraction" as it occurred after the S velocity. The presence of "post-systolic contraction" in these four PHT infants prevented IVRT measurement. Of note, a similar positive velocity, after the S wave, was also present in a further nine infants in the PHT group and also in eleven infants in the control group. In these cases however, the velocity was small and its commencement was distinct from the end of the S wave, therefore IVRT could still be measured.

Figure 7.1: PWTDI waveform from an infant with PHT



PWTDI waveform from control infant (left) and PHT infant (right). No E' velocity is present in the PHT infant; instead only a single A' velocity occurring after the P wave of the accompanying ECG. Also present in the PHT infant is a prominent post systolic positive velocity (*) after the S wave in early diastole.

RV IVRT was therefore measured in 11 infants in the PHT group, and in all 25 infants in the control group. IVRT in the IVS was measured in all infants in both groups.

In view of these difficulties measuring IVRT in the RV in four infants in the PHT group, it was decided to measure another diastolic time interval, which has not been described before and which for the purposes of this study was referred to as the diastolic myocardial displacement time (DMDT). As discussed in Chapter 2, DMDT was defined as the period from the beginning of the E' wave to the end of the A' wave. The sum of IVRT and DMDT represents the total duration of diastole. Measurement of DMDT was not affected by the presence of a "post-systolic" contraction velocity, and could be obtained in all infants in the PHT and control groups.

7.3 Systolic PWTDI velocity data

Systolic PWTDI velocities (IVV and S) were measured in the RV and IVS.

Velocities for each position, in each group, are reported in Table 7.1.

Table 7.1: Systolic PWTDI velocities in the RV and IVS

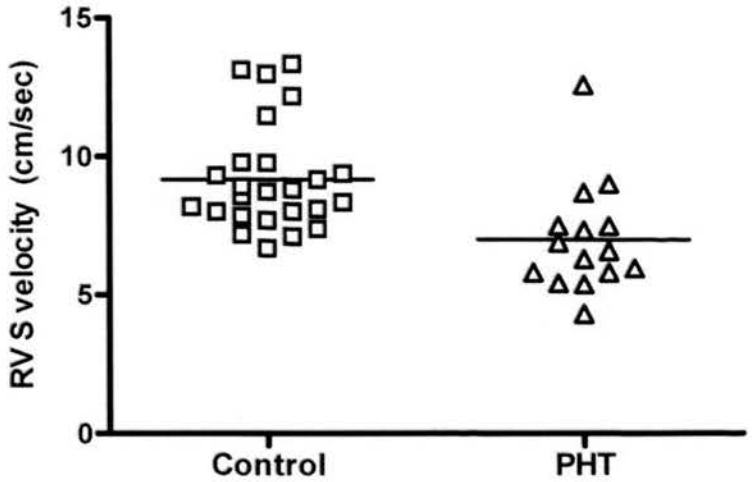
	Control Group	PHT Group	P
RV			
IVV	6.6 (1.1)	5.3 (1.3)	0.0015
S	9.2 (1.9)	7.0 (2.0)	0.0004
IVS			
IVV	3.4 (0.5)	3.9 (1.1)	0.19
S	5.0 (0.7)	5.1 (1.4)	0.95

All data mean (SD)

7.3.1 Systolic PWTDI velocities in the RV:

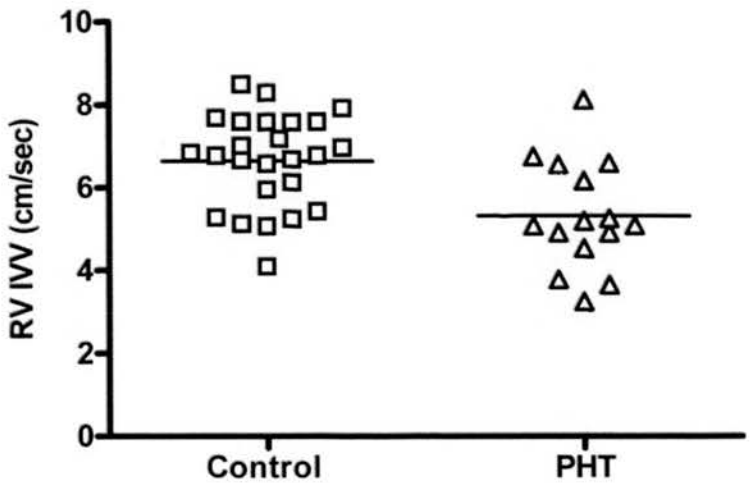
In the RV, IVV was significantly reduced in the PHT group compared to the control group (5.3 vs. 6.6 cm/sec, P=0.0015). S velocity was also significantly reduced in the RV in the PHT group compared to the control group (7.0 vs. 9.2 cm/sec, P=0.0004). These between-group differences are demonstrated graphically in Figures 7.2 and 7.3.

Figure 7.2: RV isovolumic contraction velocity (IVV) in PHT and control groups



Lines represent means. P=0.0015.

Figure 7.3: RV S velocity in PHT and control groups



Lines represent means. P=0.0004

7.3.2 Systolic PWTDI velocities in the IVS

In the IVS there was no significant between-group differences in IVV (3.9 [1.1] vs 3.4 [0.5] cm/sec, P=0.19) or S velocity (5.1 [1.4] vs. 5.0 [0.7] cm/sec, P=0.95). Of note, systolic velocities were significantly lower in the IVS than in the RV, in both the control and PHT groups.

7.4 Diastolic PWTDI velocities

Diastolic PWTDI velocities (E' and A') in the RV and IVS are summarised in Table 7.2.

Table 7.2: Diastolic velocities in the RV and IVS, in PHT and control groups

	Control Group	PHT Group	P
RV			
E'*	-8.6 (2.0)	-4.1 (2.2)	0.0006
A'	-11.7 (4.2)	-10.1 (2.5)	0.21
IVS			
E'*	-5.4 (1.1)	-3.8 (0.5)	<0.0001
A'	-6.3 (1.3)	-6.4 (2.9)	0.73

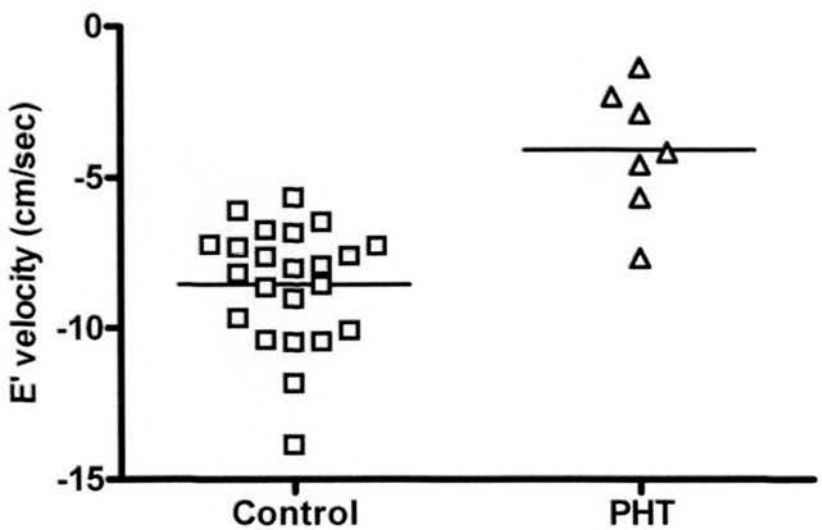
*E' velocity from 7 infants in PHT group in whom E' velocity was present and measurable. In remaining 8 infants in PHT group no E' velocity was present and no velocity measured.

7.4.1 Diastolic PWTDI velocities in the RV

E' velocities were present in the RV of all infants in the control group with a group mean (SD) of -8.6 (2.0) cm/sec. E' velocities were present in the RV of seven infants in the PHT group. In the remaining eight infants in the PHT group no E' velocity could be identified. No E' velocity was recorded in these infants. This issue is discussed in detail later in the chapter and all numerical PHT group data presented here excludes these infants.

Mean RV E' velocity for the entire PHT group was -4.1 (2.2) cm/sec. The reduction in E' velocity in the PHT group compared to the control group was highly significant (P=0.0006). This inter-group difference in represented graphically in Figure 7.4.

Figure 7.4: RV E' in PHT and control groups



Lines represent means. P=0.0006

A' velocity in the RV in the control group was -10.1 (2.5) cm/sec, and in the PHT group was -11.7 (4.2) cm/sec. There was no significant inter-group difference, P=0.21.

7.4.2 Diastolic PWTDI velocities in the IVS

Diastolic velocities in the IVS are provided in Table 7.2, and discussed here. E' velocity in the IVS in the control group was -5.4 (1.1) cm/sec. In the PHT group no IVS E' wave was present in seven infants, and in these cases no E' velocity was recorded. These were the same infants who had no identifiable E' wave in the RV. Mean E' velocity in IVS in the PHT group was -3.8 (0.5). The reduction in E' velocity in IVS in the PHT group was significant (P<0.0001).

A' velocity, in the IVS, in the control group was -6.3 (1.3) and was not significantly different from A' velocity in the PHT group; -6.4 (2.9), P=0.73.

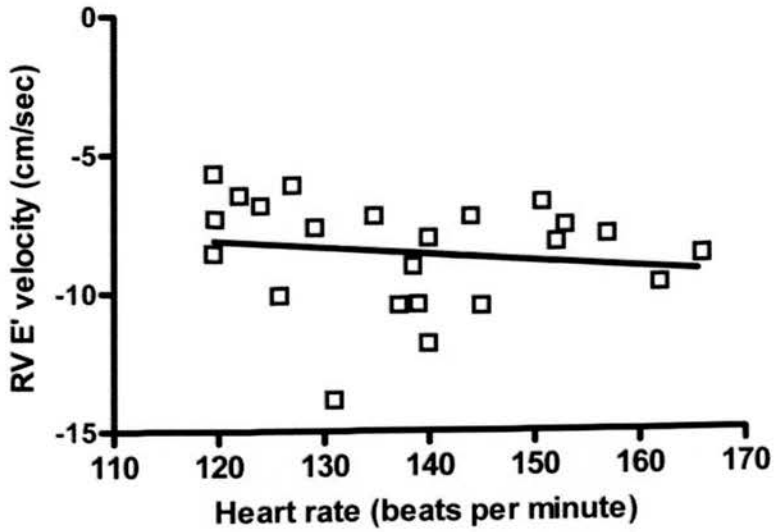
It is notable that E' and A' velocities were of significantly lower amplitude in the IVS than in the RV, in the control group (P<0.0001).

7.4.3 E' velocity and heart-rate

Diastole is shortened at higher heart rates. It was therefore considered that E' velocity may also be altered by heart rate, and that heart rate differences between the PHT and control groups might have contributed to the observed inter-group differences in RV E' velocity. The relationship between E' velocity was therefore

investigated. As shown in Figures 7.5, there was poor correlation between E' velocity and heart rate in the control group (r^2 =0.03, P=0.44).

Figure 7.5: Relationship between RV E' velocity and heart-rate in control group

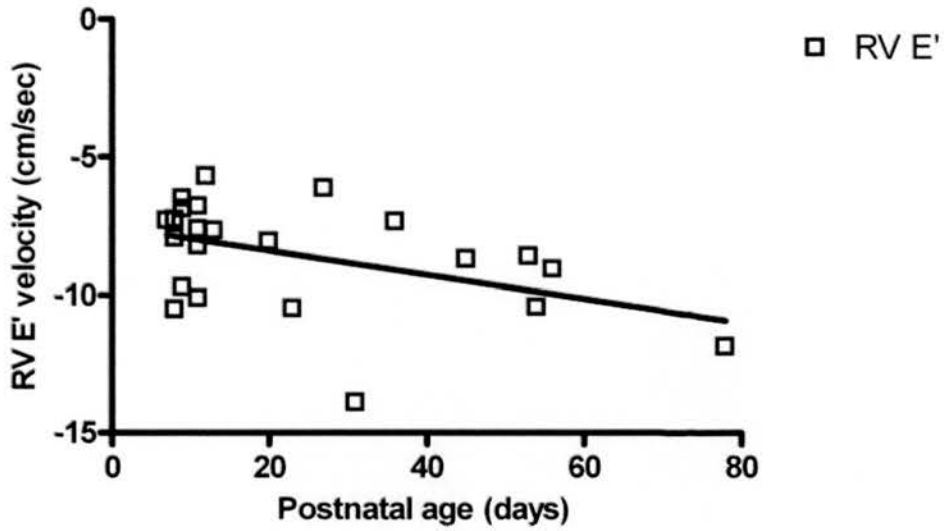


r^2=0.03, P=0.44

7.4.4 Diastolic velocities and age

RV diastolic function is known to change in the first days of postnatal life [67]. The relationship between RV diastolic velocities and postnatal age was therefore investigated. In the control group RV E' velocity was found to correlate negatively with postnatal age (r^2=0.2, P=0.03) as shown in figure 7.6. RV A' velocity in the control group did not show significant correlation with age (r^2=0.1, P=0.1).

Figure 7.6: RV E' velocity and postnatal age in the control group



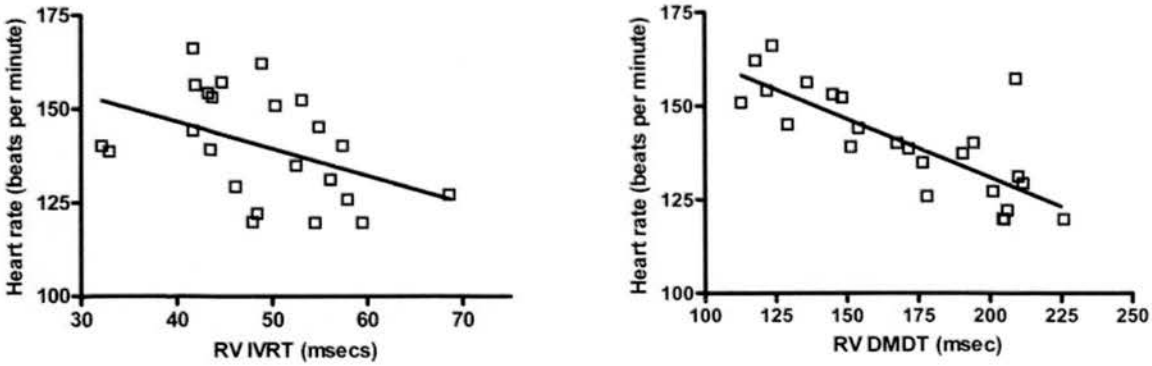
Control group: r^2=0.2, P=0.03

7.5 Diastolic time intervals

IVRT and DMDT were measured from the RV and IVS PWTDI waveforms of infants in each group. As discussed above, IVRT could not be measured in the RV in four infants in the PHT group due to the presence of positive "post-systolic" velocities.

Time intervals were corrected for heart rate by dividing the time interval by the heart rate (R-R interval) to produce a ratio i.e. IVRT:RR and DMDT: RR. This correction was deemed valid based on the observation that, in the control group, both IVRT and DMDT demonstrated good linear correlation with heart rate (Figure 7.7).

Figure 7.7: Relationship between diastolic time intervals and heart rate in control group



r^2=0.18, P=0.04

r^2=0.62, P<0.0001

Corrected diastolic time intervals in the RV and IVS are listed for control and PHT groups in Table 7.3. IVRT:RR was significantly *increased* in the PHT group compared to controls in the RV (0.22 [0.04] vs. 0.11 [0.02], P<0.0001) and in the

IVS (0.22 [0.05] vs. 013 [0.02], P<0.0001). DMDT:RR was, accordingly, significantly *reduced* in the PHT group compared to controls, both in the RV (0.30 [0.07] vs. 0.39 [0.05], P=0.0001) and in the IVS (0.31 [0.08] vs. 0.37 [0.04], P=0.035).

Table 7.3: Diastolic time intervals in the RV and IVS in PHT and control groups

	Control Group	PHT Group	P
RV			
IVRT:RR	0.11 (0.02)	0.22 (0.04)	<0.0001
DMDT:RR	0.39 (0.05)	0.30 (0.07)	0.0001
IVS			
IVRT:RR	0.13 (0.02)	0.22 (0.05)	<0.0001
DMDT:RR	0.37 (0.04)	0.31 (0.08)	0.035

7.6 Discussion

7.6.1 Feasibility

These data confirmed the feasibility of performing PWTDI in the RV and IVS of newborn infants. The technique was well tolerated and safe. Although PWTDI data was not collected in three infants in the control group, this was not due to any difficulty performing the technique. Instead, studies were cut short for unrelated clinical reasons. This highlights the limitations of any study in sick newborn infants

who require ongoing routine clinical care, which may disrupt collection of study data.

These data also demonstrate the feasibility of post-acquisition analysis of PWTDI data to obtain myocardial velocities and time intervals. This included analysis of PWTDI data from three infants receiving HFOV and three receiving HFJV. In one infant in the PHT group however, HFOV did produce artefactual disruption of the PWTDI waveform preventing meaningful analysis. The reasons why artefact was encountered in the PWTDI waveform in this subject, and not in the others receiving high frequency ventilation, were unclear. However, it is hypothesised that increased compliance of the lungs and chest wall, in combination with the ventilation settings used, may have allowed greater transmission of oscillations from the airways to the ultrasound transducer.

7.6.2 PWTDI velocities in the control group: comparison with previous studies

PWTDI analysis of myocardial velocities in newborn infants has previously been performed in two studies, Mori et al [94] and Pateman et al [105]. Mori et al measured S, E' and A' velocities, in the RV, IVS and LV, in a group of term infants aged between one and seven days (n=135). Pateman et al, meanwhile, measured PWTDI velocities in the IVS (S, E' and A') in 45 preterm infants, 32 of whom had patent arterial ducts. PWTDI velocities obtained in these prior studies, together with study group demographics, are shown for comparison with control group data from the current study, in Table 7.4.

Table 7.4: PWTDI velocities in control group and prior studies

	Mori et al [94]	Pateman et al [105]	Control group
N	135	45	28
Weight	2.95 (0.3)	2.1 (1.0)	3.2 (0.7)
Gestation	39.5 (1.6)	32 (6)	37.0 (3.5)
Age	3.9 (2.0) days	16 (20) days	25 (20) days
Patent ductus	Not reported	32 (71%)	0 (0%)
RV			
S	6.6 (1.2)	-	9.2 (1.9)
E'	-7.5 (1.3)	-	-8.6 (2.0)
A'	-9.2 (1.5)	-	-10.1 (2.5)
IVS			
S	3.9 (0.7)	4.2 (1.2)	3.4 (0.5)
E'	-5.4 (0.9)	-5.9 (1.5)	-5.4 (1.1)
A'	-5.2 (0.9)	-3.7(0.7)	-2.2 (2.0)

PWTDI velocities obtained in the control group were generally in good agreement with those reported in the work of Mori et al and Patemen et al. Of note, however, RV S wave velocity tended to be higher in the current study control group than in the study of Mori et al (mean velocity 9.2 vs. 6.6 cm/sec). The higher RV S velocity in the current study may be related to age differences between the two different study populations. In the study of Mori et al, S velocity in the RV positively correlated with the postnatal age (in days). The control group, from which the current data were obtained, were considerable older than the group of Mori et al (25 days vs 3.9 days), and might therefore be expected to have higher S wave velocities.

Septal A' velocity tended to be lower in the control group compared to the data of Mori et al (-2.2 vs -5.2 cm/sec). The lower IVS A's wave in the current control group, compared to the data of Mori et al, cannot easily be explained by any age differences between the study populations, as no correlation was observed between A' velocity and age.

Gestation and ductal patency may conceivably alter PWTDI velocities and may have contributed to any differences in PWTDI velocities between these and the current study. The study population in the work of Pateman et al was preterm (mean gestation 32 weeks) and also had a high frequency of PDA (71%). The frequency of PDA in the study of Mori et al was not reported, but since these infants were between one and seven days of age it is possible that a number may have had a patent arterial duct.

The current study is the first to measure IVV in a newborn population, as this was not measured in either the study of Patemen et al or Mori et al. IVV has been previously validated as a non-invasive measure of systolic contractile function in animal and adult studies [49, 82]. The current study therefore provides the first normative reference data (from the control group) in an infant population.

In the control group PWTDI velocities were noted to be lower in the IVS than in the RV in all phases of the cardiac cycle. This replicates the finding of Mori et al [94]. Higher velocities in the RV have previously been attributed to lower after-load on the RV (compared to LV and IVS after-load) [79].

7.6.3 Correlation of E' velocity and age

E' velocity, in the control group, increased in magnitude with age, in the first three months of life. Changes in RV diastolic function in the first weeks of life have previously been demonstrated by tricuspid valve Doppler studies, and may reflect the transition from a dominant fetal RV to the adult RV [55, 67]. Interestingly, Mori et al found no such relationship between RV E' velocity and postnatal age, but studied infants in only the first week of life. This may have been too short a timescale to observe significant change in PWTDI velocities.

Could inter-group differences in E' velocity be explained by postnatal age differences? The control group were older than the PHT group (mean 25 vs. 17 days; P=0.05), and therefore the lower E' velocities in the control group may be, at least in part, attributable to this age difference.

7.6.4 Reduced systolic and diastolic PWTDI velocities in PHT: significance and comparison with previous reports

Systolic velocities

This study is the first to use PWTDI to investigate systolic function in infants with PHT and demonstrated significantly reduced IVV and S velocities in this population. Similar findings have been reported in an adult study by Moustapha et al which employed PWTDI to investigate RV function in PHT [93]. This group observed reduced S wave velocity in a group of 90 adult patients with chronic pulmonary hypertension, compared to 50 normal adult controls. IVV was not reported.

What is the significance of reduced IVV and S velocities in RV in PHT? As discussed in the background chapter, S wave velocity correlates well with conductance catheter measures of systolic function [80], but also appears to demonstrate some dependence on preload and afterload [49]. The observed reduction in S velocity may therefore indicate impaired systolic contractile function and/or the direct effects of increased afterload in PHT. IVV, meanwhile, appears to be a more robust measure of contractile function [82] which is only affected directly by extreme loading conditions [49]. The finding of reduced IVV in the RV in PHT group therefore provides stronger evidence of a genuine reduction in systolic contractile function in the infants with PHT. This finding highlights the potential of PWTDI as a non-invasive technique for assessing and quantifying systolic function, independent of diastolic function, in newborn infants.

It is of note that, despite the reduction in mean systolic velocities in the PHT group, there was overlap of the ranges of systolic velocities between the two groups, as represented visually in Figures 7.2 and 7.3. The preservation of "normal" velocities in some infants in the PHT group likely reflects the variability of RV function in PHT, which is recognised clinically, but has not been formally reported in infants before [35].

Diastolic velocities

The reduction, or loss, of distinct E' velocities in the PHT group (in both the RV and IVS) was one of the most striking findings of this study. Indeed in eight infants in

the PHT group E' velocities were entirely absent in the RV. Three possible explanations for the absence of E' velocities in these infants were considered:

1. E' velocities were reduced to the extent that they were undetectable on PWTDI analysis (i.e effectively zero).

2. E' velocities were present but were delayed and occurred later in diastole at the same time as the A' velocity. (and were therefore "hidden" within the A' wave).

3. E' velocities were present but were fused with A' velocities as a result of higher heart rates in the PHT infants.

The first explanation was considered the most likely on the basis that in the remaining eight infants E' velocities *were* present, preceding A' velocities, but reduced compared to control infants. A delay in E' velocity, leading to fusion with A' velocities, was possible but was not observed in the other infants with PHT who did have an E' velocity present, making this a less likely explanation. Considering the third explanation; although heart-rate in the PHT group was increased compared to the control group E' velocity correlated poorly with heart-rate in both the PHT and control groups suggesting that higher heart rates were not responsible for lower E' velocities. Furthermore, even when maximal Doppler sweep speeds (150mm/sec) were used, to separate out myocardial velocities as much as possible, no discernible E wave could be identified in those 8 infants.

In view of these considerations, the absence of an identifiable E' wave in eight PHT infants was believed to represent the genuine absence of a detectable early diastolic myocardial velocity. This was one of the most striking findings of this study. However, as no E' velocity could be measured in these eight infants they were not included in statistical comparison between the PHT and control groups.

Although PWTDI has not been previously employed to assess RV function in infants with PHT, similar findings have been observed in adults with PHT. Moustapha et al reported that E' velocities, in the lateral (RV) and medial (IVS) tricuspid valve annulus, were significantly reduced in adults with PHT compared to healthy controls, whilst A' velocities were unchanged [93].

What is the mechanistic significance of reduced, or absent E' waves in PHT infants? As discussed in Chapter 2, E' velocities correlate with invasive measures of active myocardial relaxation [27, 89]. The reduction in E' velocity in PHT may therefore indicate impaired active myocardial relaxation in early diastole. In those cases where no E' velocity was present it appears that early active diastolic relaxation was entirely absent. It is hypothesised that in the dilated, pressure-loaded RV, operating beyond the peak of the Starling curve, the contractile apparatus of the myocytes is stretched to the point that further active relaxation is not possible and instead ventricular filling, and myocardial displacement is dependent on atrial contraction.

Diastolic time intervals

IVRT was lengthened in infants with PHT, and DMDT correspondingly shortened. Lindqvist et al have similarly demonstrated prolongation of PWTDI derived IVRT in the RV of adults with PHT. IVRT is known to correlate with invasive measures of myocardial relaxation [27], but also appears to be directly load-dependent [91]. Prolonged IVRT in the PHT group may therefore represent impaired relaxation and/or the direct effects of increased afterload.

Post-systolic velocities

The finding of post-systolic positive velocities, occurring after the S wave and before the E' or A' velocity, was of interest. These were present in 11 infants in the control group (in whom they did not interfere with IVRT measurement). It was therefore hypothesised that they were a normal finding and may represent re-configuration of the ventricle, i.e. relaxation in some parts of the ventricle and contraction in other parts, in the transition from the contracting, shrinking systolic ventricle to a relaxing, expanding diastolic ventricle. In four infants in the PHT group, however, the post-systolic velocities were of high velocity (approximately equal to S') and continuous with the S' wave preventing measurement of IVRT. It was considered that in these infants the exaggerated post-systolic positive velocities might reflect ongoing contraction of the pressure-loaded ventricle after pulmonary valve closure, or discoordinate, asynchronous relaxation of the failing ventricle. This unexpected observation represents a potential area for further study. Interestingly, similar findings have been described in the failing adult LV where it has been noted that impaired relaxation (i.e. diastolic dysfunction) is characterised by a prolonged IVRT

secondary to asynchronous relaxation of different parts of the diseased myocardium [26].

7.6.5 Limitations of this study

There were limitations inherent in this study at the stages of data collection, analysis and interpretation. Limitations relating to the study population have been dealt with previously (Chapter 3). At the time of collection of pulse wave data there may have been variability in the positioning of the pulse wave Doppler sample volume within the myocardium. Care was taken to use the smallest sample volume (2mm) and position this within the desired region of basal myocardium in the RV or IVS. However, any movement of the heart, chest wall, or ultrasound probe may have led to mal-placement of the sample volume and could have altered the PWTDI velocities obtained. Care was also taken to ensure that the angle of incidence of the Doppler beam was less than 15^{o}. Any increase in this angle of incidence might have led to underestimation of PWTDI velocities. This was a particular issue in infants in whom the heart is displaced within the thorax (e.g. CDH). It must also be noted that PWTDI velocities were only measured in the longitudinal plane (along the line of the Doppler beam), whilst true maximal myocardial velocities may have occurred in another direction, or combination of directions.

The observer was not blinded to PHT or control group status during data collection or analysis, nor was intra-observer variability assessed. Furthermore, previous studies using PWTDI in infants have reported intra-observer variability for

measurement of S, E' an A' velocities of 5.2 (3.0)%, 5.0 (3.6)%, and 5.5 (3.6)% respectively [94].

Finally, PWTDI velocities were measured only in the basal region of the ventricle. Velocities may have been different in other myocardial regions, and shown different changes in the PHT group. The timing or synchrony of myocardial velocities was also not studied, but may be altered in disease states such as PHT [136, 137].

7.6.6 Limitations of PWTDI

PWTDI, though feasible in infants, has a number of limitations. Standard ultrasound machines available in clinical neonatal practice may not have the ability to perform this technique, although most new machines are fitted with the filters capable of allowing PWTDI. The technique is also moderately time consuming. Although formal timing was not performed in the current study, the separate collection of data in each myocardial region of interest, and subsequent analysis of individual velocities, may take too long for practical use in the clinical setting,

It was not possible to use PWTDI to measure IVA in infants due to the limitations of spectral broadening, pulse frequency rates and sweep speeds. These are limitations of PWTDI technology in its current form. Instead only IVV and S can be assessed in infants as measures of systolic function. Diastolic function may be assessed using E' velocity and IVRT, but, as discussed, these may also demonstrate varying degrees of load-dependence and cannot be considered "pure" measures of diastolic relaxation

[27]. No known PWTDI parameter measures the other determinant of diastolic function - passive chamber stiffness.

7.7 Conclusions

This study has demonstrated the feasibility of PWTDI as a measure of RV function in control and PHT infants. Importantly, PWTDI allows separate assessment of systolic and diastolic function. This study provided normative PWTDI velocity data, which is in good agreement with prior studies. Use of PWTDI in this study demonstrated, for the first time, both systolic and early diastolic myocardial dysfunction in infants with PHT compared to controls. The important incidental finding of post-systolic positive myocardial velocities in both control and PHT groups represents an area for further study.

CHAPTER 8

COLOUR TISSUE DOPPLER IMAGING

8.1 Introduction

This chapter presents and discusses the data relating to use of CTDI to assess RV function in control and PHT infants. CTDI was the most advanced method of ventricular function assessment employed in this work, and has not been previously reported in infants. This study was therefore an important feasibility assessment of CTDI in infants, with the potential also to provide more detailed information about ventricular function than any of the other techniques used.

CTDI data collection and analysis are discussed first, including practical difficulties encountered. The CTDI myocardial velocities and accelerations are then presented. Group summary statistics are provided and inter-group differences represented graphically where relevant. The relationships between diastolic velocities and age are described as these had the potential to contribute to any inter-group differences. In the discussion section the study findings are considered in relation to the stated aims and hypotheses, the feasibility of CTDI in infants is reviewed, and the mechanistic implications of inter-group differences discussed. Limitations of this study and of CTDI as a measure of RV function are discussed.

8.2 Data collection and analysis

CTDI data was acquired, using the techniques described in Chapter 2, in 28 infants (100%) in the control group and 15 infants (94%) of infants in the PHT group. CTDI data was not collected in the one remaining infant in the PHT group because the study was discontinued to allow ongoing clinical care of the infant. There were no

clinically significant deteriorations during any CTDI data acquisition. Although formal timings were not performed, it was the researcher's experience that the steps required to collect CTDI data from a four chamber apical view, (i.e. colourising the two-dimensional image, and recording a cine-loop of 5 cardiac cycles) could usually be performed within twenty seconds.

CTDI analysis was a more cumbersome and time-consuming process, as has been previously described in Chapter 2. The process is briefly described again here, to indicate the steps involved and duration. After data acquisition, offline CTDI analysis was performed using an additional software program (Qlab^{TM}). Within Qlab the Doppler sample volume was positioned in the region of myocardium for analysis (region of interest), at the beginning of each cineloop. The sample volume width and length were then adjusted to the specifications for the study. Next, the cineloop was played slowly and the sample region position adjusted manually, during the entire cineloop recording, to maintain its position in the desired region of interest (i.e. manual tracking of myocardial segment). A waveform of CTDI velocities in the region of interest was produced in this way, from which systolic and diastolic velocities could then be measured manually over five consecutive cardiac cycles. This process was repeated to obtain myocardial velocities in both the basal RV and interventricular septum. This multi-step process of CTDI analysis took approximately twenty minutes per infant.

CTDI analysis was performed in all 28 infants (100%) in the control group. In two infants in the control group distinct E' velocities could not be identified.

CTDI analysis was completed in all infants in the PHT group. E' waves were absent in the RV in 10 of these infants, and in the interventricular septum in five of the infants. In these cases no E' velocity was recorded nor included in statistical analysis. Furthermore, in two infants in the PHT group a separate isovolumic contraction velocity (IVV) could not be identified and therefore IVV and IVA could not be quantified.

A significant problem, identified in analysis of data from all infants, related to the limited sampling frequency of the software used. The sampling frequency is the rate at which myocardial velocities were sampled, and was determined by the frame rate at which CTDI was acquired and the heart rate of the infant. The frame rates, heart rates and calculated sampling frequency (frames per heart beat, i.e.samples per cardiac cycle) are provided for each group in Table 8.1 below.

Table 8.1: Heart rate, CTDI frame rate and calculated sample frequency

	Heart rate (bpm)	**Frame rate (Hz)**	**Sample frequency* (Frames per heart beat)**
Control Group mean (range)	141 (120-186)	182 (168-213)	77.5
PHT group mean (range)	153 (130-194)	179 (137-212)	70.2

* Sampling frequency = frame rate / (HR/60)

There was no statistical difference between heart-rate in the control and PHT groups; 141 (19) vs. 153 (18) beats per minute, P=0.06. The average sampling frequency, in both the control and PHT groups was only approximately 70 samples per cardiac cycle.

This limited sampling frequency had a number of important implications for data analysis:

- Sample points may not have corresponded to true peak velocities. Measured myocardial velocities (corresponding to the "peak" sampled velocity) may have been lower than the true maximum velocities. This was particularly so for IVV which was a very brief velocity, with a short-lived peak. Any such invalidation of IVV measurement would also invalidate IVA (the slope of IVV).

- There was loss of the characteristic CTDI waveform and its component velocities (IVV, S, E' and A') in some infants. It was hypothesised that insufficient sampling frequency had led to "fusion" of velocities in these infants. The presence of a simultaneous ECG was therefore essential to aid identification of velocities according to their timing in the cardiac cycle.

- Time intervals (IVRT and DMDT) could not be accurately measured in many infants. Measurement of time intervals could only be made between sample points in the myocardial velocity waveform. As sample points did not always correspond to the beginning and end of the specified time intervals, these intervals could not be accurately measured using the analysis software.

These issues, though significant, were not considered sufficient to completely invalidate all subsequent CTDI data analysis. However, potential inaccuracy in peak CTDI velocities measurement was taken into account in subsequent interpretation of the data.

8.3 Systolic CTDI velocities and accelerations

Systolic CTDI velocities obtained in the RV and septum of infants in the control and PHT groups are summarised in Table 8.2 below.

Table 8.2: Systolic CTDI velocities and acceleration in control and PHT groups

	Control	PHT	P
RV			
IVA*	68.1 (30.2)	95.5 (31.6)	0.003
IVV*	1.3 (0.6)	1.3 (0.5)	0.641
S	2.6 (0.8)	2.1 (0.7)	0.0855
Septum			
IVA	72.0 (22.4)	87.2 (33.3)	0.09
IVV	0.8 (0.4)	1.2 (0.9)	0.187
S	2.1 (0.4)	1.5 (0.4)	<0.0001

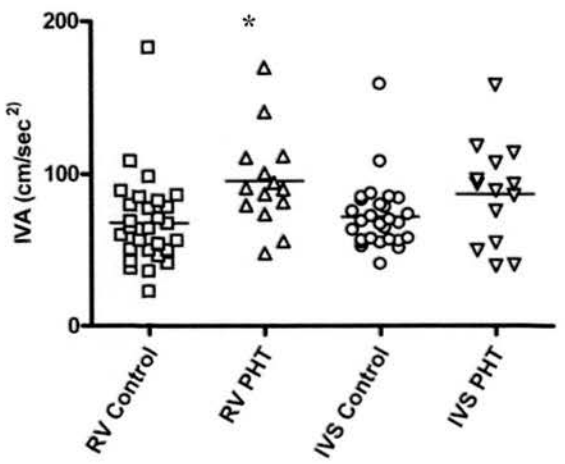
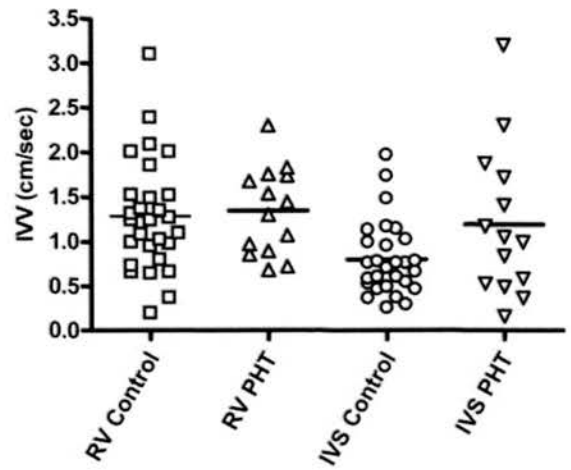
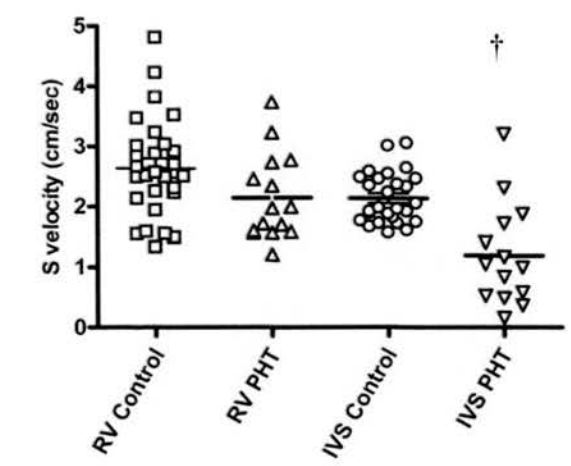
All data mean (SD). Units are cm/sec (IVV and S) and cm/sec^2 (IVA). *IVV and IVA not obtained in RV of 2 infants in PHT group due to failure to identify distinct IVV.

RV IVA was significantly increased in the PHT group compared to controls [95.5 (31.6) vs. 68.1 (30.2) cm/sec^2, P=0.003]. However, IVV and S in the RV, were not significantly different between the two groups.

Septal myocardial velocities tended to be lower than those in the RV. Septal IVA and IVV were not significantly altered in the PHT group compared to controls. However, septal S velocity, was significantly reduced in the PHT group; 1.51 (0.43) vs. 2.13 (0.42) cm/sec, P<0.0001.

It is notable that all CTDI systolic measures demonstrated wide variation, with large standard deviations. The systolic function data is represented graphically in Figure 8.1 and provides an appreciation of the degree of variance within each measure.

Figure 8.1: IVA, IVV and S velocities in PHT and control groups

	IVA in RV and interventricular septum (IVS). IVA was significantly increased in the RV in PHT infants compared to controls (*)
	IVV in the RV and IVS. There were no significant differences in IVV, in the RV or IVS, between PHT and control groups.
	S velocity in the RV and IVS. S velocity was significantly reduced in the IVS in the PHT group compared to the Control group (†).

8.4 Diastolic CTDI velocities

Diastolic CTDI velocities, obtained in the RV and IVS, are summarised in Table 8.3 below.

Table 8.3: Diastolic CTDI velocities in the RV and interventricular septum

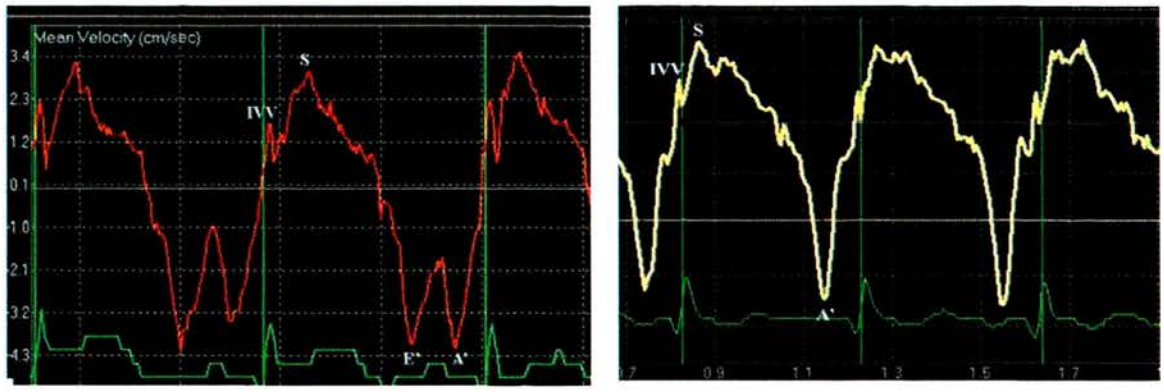
	Control	PHT	P
RV			
E' * (n=5)	-3.3 (1.0)	-2.4 (1.6)	0.081
A'	-3.4 (1.7)	-4.1 (1.7)	0.170
Septal			
E' * (n=10)	-3.1 (0.7)	-1.8 (0.5)	<0.0001
A'	-3.5 (0.9)	-3.2 (1.0)	0.314

All data mean (SD). *E' velocity absent in RV of 10 infants in PHT group, and absent in the interventricular septum of 5 infants in the PHT group; No E' velocity recorded in these infants and data therefore not included in the statistical analysis presented here.

E' velocity (where present) was not significantly reduced in the RV in the PHT group (-3.3 [1.0] vs. -02.4 [1.6] cm/sec, P<0.081). However, E' velocity was significantly reduced in the interventricular septum in PHT infants (-3.1 [0.7] vs. -1.8 [0.5] cm/sec, P<0.0001). A' velocity was not significantly different in the PHT compared to controls, in either position.

Sample CTDI waveforms from a control infant and a PHT infant are presented in Figure 8.2, providing graphical demonstration of the loss of the E' wave in some ten PHT infants.

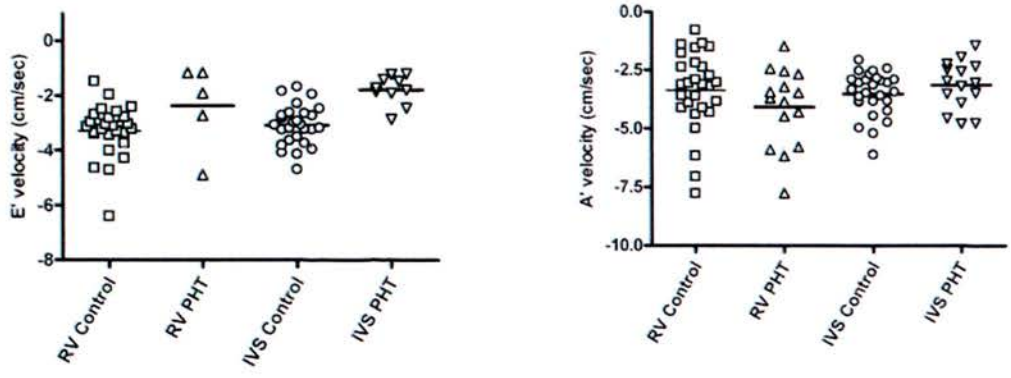
Figure 8.2: CTDI waveforms in control and PHT infants



CTDI waveforms from control (left) and PHT (right) infants. All systolic (IVV, S) and diastolic (E', A') velocities identified in control infant. In PHT infant no E' velocity diastolic velocity can be identified.

The variance in CTDI diastolic velocities, in both PHT and control groups, was broad. This can be observed from the large standard deviations of each velocity, and from the graphical representation of individual infant data plotted in Figure 8.3.

Figure 8.3: E' and A' velocities in the RV and interventricular septum



Line represents mean. Data from PHT infants in whom no E' velocity present (ten infants in RV, 5 infants in IVS) not included in this figure.

8.5 Diastolic velocities and age

As considered in Chapters 4 and 7, diastolic function may change in the RV following birth. The relationship between control group diastolic CTDI velocities (in the RV) and postnatal age was therefore investigated. There was no significant correlation between postnatal age and either RV E' velocity ($r^2 = 0.002$, P=0.83) or RV A' velocity (r^2=0.08, P=0.14).

8.6 Discussion

8.6.1 Feasibility

This study has shown that the *collection* of CTDI data could be performed rapidly and safely in infants. However, the *analysis* of CTDI data was less straight-forward and raised questions as to the feasibility of the technique.

Analysis was a time consuming process which involved multiple steps to obtain a velocity waveform and manually measure velocities from this. Furthermore, there were major concerns that measurement error was introduced due to the limitations of the sampling frequencies used. Even though the frame rate was optimised to maximise sampling frequency, it was felt the CTDI software did not sample frequently enough to ensure that maximal myocardial velocities were accurately detected. This was considered to have also led to distortion of the normal appearance of the myocardial Doppler waveform (which made identification of individual velocities difficult).

The wide variance observed in all CTDI measures (IVV, S, E' and A'), in both control and PHT groups, may have resulted in part from this measurement error. In some infants the measured velocity may have been close to the true maximum (if the sample point occurred at true peak velocity) whilst in other infants the measured velocity may have been under-measured (if the sample point fell outwith the true peak).

These issues highlighted limitations of the CTDI acquisition and analysis software used and brought into question the validity of the CTDI data obtained in this study. This was borne in mind when interpreting the study data as discussed below.

8.6.2 CTDI velocities in the control group: comparison with previous studies

There are no prior published reports of CTDI velocities, or accelerations (IVA), in newborn infants for comparison with the current data. In the absence of comparable data, and in light of the concerns of measurement error, the control group data cannot be recommended to represent normative standard data.

Normative CTDI data has previously been obtained in healthy children by Pauliks et al [138], and in healthy adults by Kjaergaard et al [74]. The CTDI velocities and accelerations obtained in the RV in these studies, and in the control group of the

current study, are listed in Table 8.4 below, together with demographic data for each study population.

Table 8.4: CTDI velocities and accelerations in control group and previous studies

	Kjaergaard et al	Pauliks et al	Control Group
Demographic data			
N	17	75	28
Age	31 (9) years	8.5 (5.1) years	25 (20) days
Weight (kg)	-	34.4 (25.4)	3.2 (0.7)
Heart rate (beats per minute)	60 (10)	86.5 (23.1)	141 (19)
Frame rate (frames/sec)	130 f/se c	>100 f/sec	182
Sample frequency (frames per heart beat)	130	69	77
CTDI data			
RV			
IVA	-	190 (60)	68.1 (30.2)
IVC	-	6.4 (2.2)	1.3 (0.6)
S	10.4 (1.3)	10.0 (1.7)	2.6 (0.8)
E'	-10.4 (3.8)	-12.1 (3.0)	-3.3 (1.0)
A'	-9.5 (3.4)	-7.6 (3.6)	-3.4 (1.7)
Septum			
IVA	-	120 (40)	72.0 (22.4)
IVC	-	2.9 (1.2)	0.8 (0.4)
S	-	6.1 (1.1)	2.1 (0.4)
E'	-	-10.2 (1.8)	-3.1 (0.7)
A'	-	-4.8 (1.6)	-3.5 (0.9)

It can be seen from Table 8.4 that the CTDI velocities obtained in infants (control group) were much lower than those obtained in either older children (Pauliks et al) or adults (Kjaergaard et al). This increase in myocardial velocities with age has previously been reported by Mori et al using PWTDI, but never before using CTDI techniques [94]. In both the control group, and the older children studied by Pauliks et al, CTDI velocities were lower in the septum than in the RV. This is again in agreement with PWTDI data from the current study (chapter 7) and previous studies [94].

The frame rates and heart rates in each study, together with the calculated sampling frequency, have also been provided in Table 13. In Kjaergaard et al's study in adults the lower heart rates allowed for a higher sampling frequency than in the control group. However, in Pauliks et al's study the calculated sample frequency was similar to that in the control group (based on a reported frame rate of 100 frames/sec). The concerns that the sampling frequency may have been too low in the control group to measure peak CTDI velocities accurately may also therefore be applicable to the data of Pauliks et al.

No significant relationship was demonstrated between E' velocity in the RV and age. This suggested that the observed differences between PHT and control groups could not be attributed to age differences between the groups. Furthermore, this has practical implications for future use of CTDI, allowing inter-subject comparison of CTDI velocities without age correction.

8.6.3 CTDI velocities in PHT: significance and comparison with previous studies

Systolic velocities

RV IVA was significantly increased in the PHT group compared to controls. IVA has previously been demonstrated to be a relatively load-independent measure of systolic function, and therefore the increase in IVA in the RV might be considered to reflect increased myocardial contractility in the PHT group. However, the other measures of systolic function, IVV and S, were not significantly different in the RV in the PHT group.

In the inter-ventricular septum, S velocity was significantly reduced in PHT, compared to controls, suggestive of impaired systolic function. If systolic function were impaired one might expect IVV and IVA also to be reduced, though in the study population they were not.

These contradictory findings were probably the result of measurement error due to the limited sampling frequency (as discussed above). This was thought to contribute to inaccuracy in the systolic velocity measures, particularly the short-lived velocity IVV (and hence IVA). Failure to identify an IVV in two PHT infants was probably due to the same issue of limited sampling frequency. In view of these concerns it was felt that no conclusions could be reached regarding systolic function differences between the groups on the basis of the CTDI data alone.

Diastolic velocities

No E' velocity could be clearly identified in the RV in ten infants in the PHT group, and in the interventricular septum in five infants in the PHT group. This was consistent with the finding of absent E' waves in the PHT group on PWTDI analysis (Chapter 7). It was not known whether the loss of E' velocities was considered to reflect either a genuine reduction in this velocity or a delay in E' velocity leading to fusion with the A' wave. A genuine reduction was considered more probable based on the observation that, in those PHT infants in whom E' velocities were present (but reduced), the E' occurred distinct from the A' wave, before the P wave on the corresponding ECG.

In those infants in whom an E' velocity was present and could be measured, it was observed that E' velocity was reduced in the PHT group compared to the control group, although this did not reach statistical significance in the RV in the PHT group.

What is the significance of a reduction in, or absence of, E' velocity in PHT? As discussed in Chapter 7, reduction in this velocity may represent either a genuine reduction in early active myocardial relaxation, and/or the direct effects of loading conditions on myocardial velocities (i.e. load dependence). There are no prior studies investigating the load-dependence of CTDI velocities in infants and it was beyond the scope of the current study to determine this. That A' velocity was unchanged in PHT suggesting that later diastolic myocardial displacement, due to atrial contraction, was not altered in PHT infants.

IVRT is another measure of diastolic myocardial function, and it had been intended to measure this in addition to CTDI E' and A' velocities. Unfortunately, it was discovered in the course of data analysis that, because of the limited sampling frequency, it was not possible accurately to measure the time points necessary to derive IVRT from CTDI data. This highlighted yet another limitation of CTDI analysis for assessment of RV function in infants.

8.6.4 Limitations of this study

Limitations related to the study population have been dealt with previously in chapter 3. The sample size was relatively small, particularly the PHT group, and this may have contributed to the failure to demonstrate consistent, statistically significant inter-group differences in systolic parameters.

In CTDI data analysis, error may have been introduced at a number of steps, including mis-identification of velocities. The CTDI waveform usually has a characteristic appearance but this was frequently distorted in the PHT group, whether due to abnormal myocardial function or the effect of insufficient sampling frequency. Although care was taken to identify CTDI velocities according to their timing in the cardiac cycle (using the ECG), it is possible that velocities may have been mis-identified and therefore the wrong velocities measured and recorded. Mal-positioning of the Doppler sample, at any time in the duration of the cine-loop, may have led to abnormal myocardial velocities being recorded from the pericardium, endocardium or ventricular cavity. Furthermore, if the angle of incidence of the

sample volume was greater than 15° this may have led to under-measurement of true peak myocardial velocities.

No attempt was made to measure intra-observer variability of the CTDI technique in this study. This was because CTDI analysis, using the hardware and software employed in this study, was time consuming and there was insufficient time available to perform the repeated analyses necessary to calculate observer variability. Pauliks et al have assessed observer variability of CTDI in normal children and obtained co-efficients of variation for systolic S velocities of 6.0% for intra-observer variability and 5.4% for inter-observer variability. For IVV the variabilities were 13.8% and 10.1% respectively, and for IVA were 10.8% and 11.5% respectively. For E' velocity the co-efficients of variation were 5.5% and 4.6% respectively, for A' 21.5% and 15.1% respectively.

In infants, user-variability might be expected to be higher for three reasons. Firstly, the absolute velocities are lower and therefore the percentage error is potentially greater. Secondly, there is greater potential for malposition of the sample volume in the smaller infant heart. Thirdly, at higher infant heart rates the limitations of sample frequency may increase the chance of mis-identification of velocities and under-measurement of peak velocities.

As in PWTDI analysis (chapter 7), CTDI velocities were obtained only in the basal RV and in the longitudinal plane. Velocities in other regions and planes of the myocardium, and the synchrony of these, were not assessed in the current study.

Further analysis of the CTDI cine-loops obtained in the study could potentially yield this information, without the requirement to repeat echocardiograms on the infants studied.

8.6.5 Limitations of CTDI

This study has highlighted major limitations of CTDI, in its current form, for assessment of RV function in infants.

Although collection of CTDI data at the infant's bedside was rapid, the analysis of the data required multiple steps which were both time-consuming and increased the potential for measurement error.

One of the most important limitations of CTDI, using the technology employed here, was the insufficient sampling frequency leading to distortion of the CTDI waveform, and inaccuracy in measurement of velocities and accelerations. Development of echocardiographic machines with higher achievable frame rates may overcome this issue.

At present CTDI analysis is only available on the most advanced, and most expensive, echocardiographic machines. In practice therefore, CTDI is not available in most neonatal intensive care units.

8.7 Conclusion

CTDI data collection was feasible in infants. There was potential for high levels of measurement error in data analysis, due particularly to insufficient sampling frequency. The latter is a limitation of current echocardiographic technology. In view of these findings, CTDI cannot be recommended for practical clinical use to assess infant RV function.

Inter-group comparison of CTDI indices failed to demonstrate consistent differences in systolic RV function. Nevertheless the absence, or reduction, of early diastolic myocardial velocities was a striking observation. The latter is in agreement with PWTDI findings in PHT.

CHAPTER 9

RELATIONSHIP BETWEEN RIGHT VENTRICULAR FUNCTION AND PULMONARY ARTERY PRESSURE

9.1 Introduction

In this chapter the data relating to the investigation of the relationship between RV function and PAP are presented and discussed. The hypothesis tested by the studies in this chapter was that, in infants with pulmonary hypertension, RV function is not linearly related to PAP.

Data collection and analysis are discussed first, outlining the paired collection of RV function and PAP data. PAP data are then presented, followed by each of the measures of RV function employed. The relationship between each measure of RV function and PAP was investigated by performing Pearson correlation analysis. Summary statistics (r^2 and P value) are reported.

In the discussion section the presence or absence of any linear correlation between PAP and each measure of RV function is discussed in relation to the stated hypothesis and the limitations of the individual measures of RV function.

9.2 Data collection and analysis

Sixty-five echocardiograms were performed in 17 infants with PHT. A maximum of eight and a minimum of two echocardiograms were performed in the same infant. Echocardiograms were performed a minimum of 24 hours and a maximum of seven days apart in the same infant.

Each echocardiographic study consisted of a paired measure of PAP and RV function. PAP was derived from peak TR jet velocity, using a modified Bernoulli equation. RV function was assessed using each of the following:

1. Transtricuspid Doppler E velocity
2. RVO
3. RV_{MPI}
4. Pulse wave TDI myocardial velocities (PWTDI)
5. Colour TDI myocardial velocities (CTDI)

Each of these echocardiographic measures was performed as previously described in Chapter 2.

9.3 Pulmonary artery pressure data

Of the 65 studies performed, a TR jet was present, from which a peak velocity could be satisfactorily measured, in only 46 studies. In the remaining 19 studies a TR jet was either absent, or a satisfactory TR Doppler waveform (from which peak TR could be identified) could not be obtained.

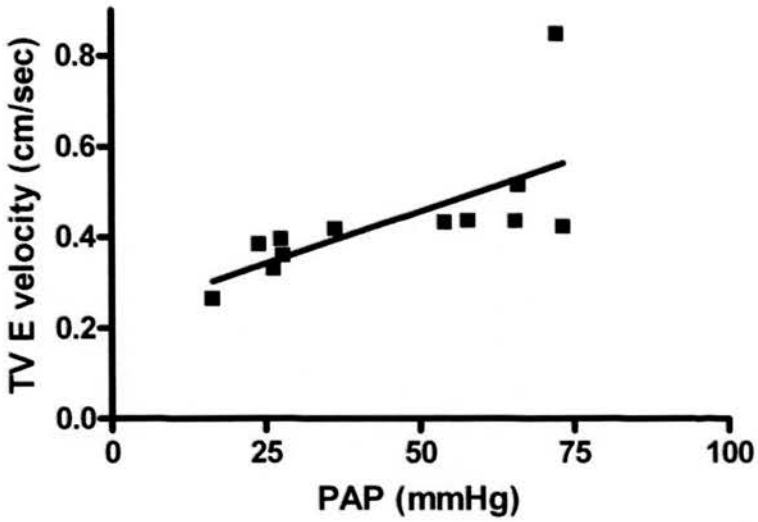
All further analysis and discussion of the relationship between PAP and RV function was limited to the 46 studies in which TR velocity (and therefore PAP) could satisfactorily be obtained. Peak TR velocity in these 46 studies was 3.65 (0.82) m/sec. The mean calculated PAP was 60.9 (22.9) mmHg.

9.4 Tricuspid Doppler E velocity and PAP

Diastolic tricuspid valve (TV) flow Doppler measurements were performed in all 46 studies in which paired recordings of PAP were made. TV E velocities were measured from the Doppler waveform, as a measure of early diastolic flow. The later TV A velocity was not included in this analysis. TV A was excluded as this is principally dependent on atrial and not ventricular myocardial function.

TV E velocity was absent in 34 studies. As discussed in Chapter 5, the absence of E velocities in some studies was a striking finding demonstrating impaired early diastolic filling in PHT. No numerical E velocity was recorded in those studies in which no E wave could be identified. Accordingly, only the 12 studies in which an E velocity was present and measurable were included in investigation of the relationship between RV function and PAP using this data. Mean TV E velocity in these 12 studies were 0.43 (0.13) cm/sec. There was a positive correlation between TV E and PAP, but with poor goodness of fit: r^2=0.46, P=0.02, Figure 9.1.

Figure 9.1: Relationship between TV E velocity and PAP



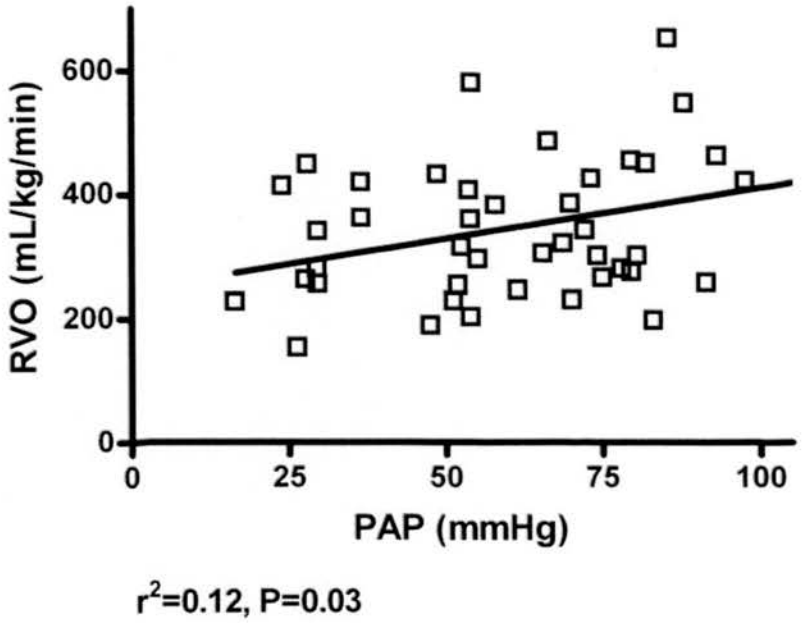
r^2=0.46, P=0.02

TV E data from 12 studies in whom E velocity present. Data not included from 34 studies in whom no E velocity present.

9.5 RVO and PAP

RVO was measured in 43 of the total 46 studies in which paired PAP measurement was made. In the remaining three studies RVO was not measured because the study was interrupted to allow ongoing clinical care. Mean RVO was 318 (114) mL/kg/min. RVO and PAP are plotted together in Figure 9.2. RVO demonstrated a significant positive correlation with PAP; r^2=0.12, P=0.03.

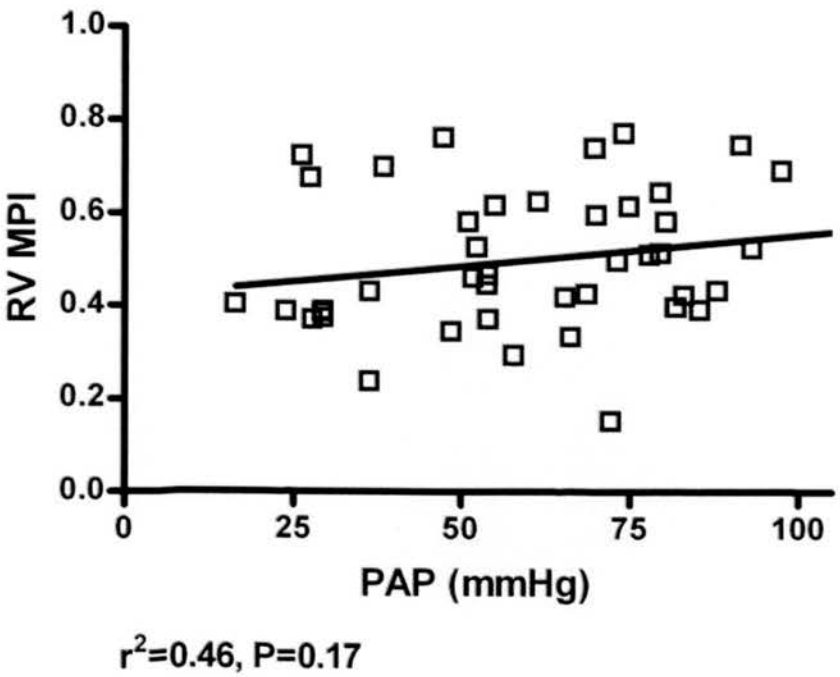
Figure 9.2: Right ventricular output (RVO) and PAP



9.6 RV_{MPI} and PAP

RV_{MPI} was obtained in 43 studies in which paired measures of PAP were also obtained. RV_{MPI} was not obtained in three studies because these were interrupted to allow ongoing clinical care. Mean RV_{MPI} was 0.51 (0.15). There was no significant correlation between RV_{MPI} and PAP; r^2=0.46, P=0.17 (Figure 9.3).

Figure 9.3: RV_{MPI} and PAP



9.7 PWTDI velocities and PAP

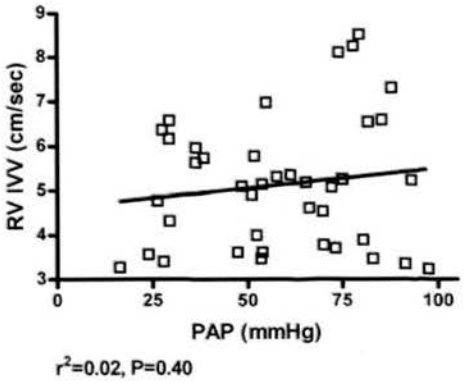
PWTDI data was obtained in 41 of the 46 studies. In the remaining five studies PWTDI data was not obtained. Three studies were discontinued before PWTDI data were obtained, to allow ongoing clinical care. In two other studies PWTDI data were obtained but velocities could not be measured due to artefact introduced by high frequency oscillatory ventilation. All data presented relate to the 41 studies in which paired PWTDI and PAP data were obtained and could be analysed. Systolic and diastolic PWTDI velocities were measured in the RV as previously discussed in Chapter 3.

9.7.1 Systolic PWTDI velocities

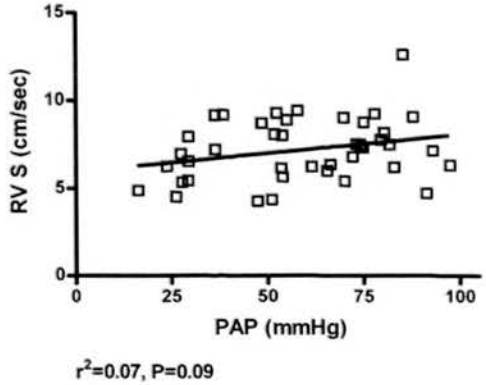
Mean RV IVV was 5.1(1.3) cm/sec. RV IVV did not significantly correlate with PAP; r^2=0.02, P=0.40, Figure 9.4, panel A. Mean RV S velocity was 7.0 (1.7) cm/sec. RV S did not correlate with PAP; r^2=0.07, P=0.09, Figure 9.4, panel B.

Figure 9.4: Systolic PWTDI velocities and PAP

A: PWTDI RV IVV and PAP



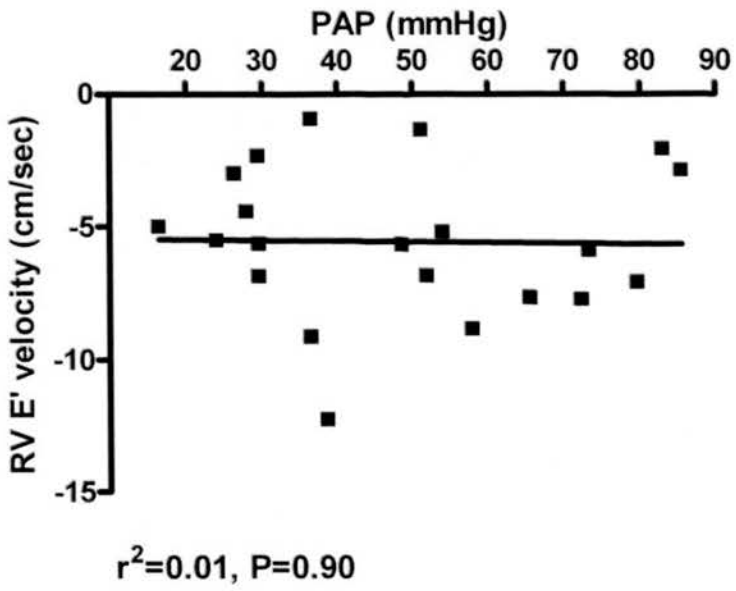
B: PWTDI RV S and PAP



9.7.2 Diastolic PWTDI velocities

E' velocities were present in 21 studies, but absent in 20 studies. It was not considered valid to include studies in which E' velocities were absent in the statistical analysis of the relationship between RV E' and PAP. Mean RV E' velocity in the remaining 22 studies was -5.5 (32.8) cm/sec. There was no significant linear correlation between RV E' velocity and PAP; r^2=0.01, P=0.90. RV E' and PAP are plotted together in Figure 9.5. The relationship between RV A' velocity and PAP was not investigated as RV A' is more dependent on atrial function than ventricular function and, as observed in Chapter 8, was unaffected in PHT infants compared to control infants.

Figure 9.5: PWTDI E' velocity and PAP



RV E' velocities from 21 studies in which E' velocity present. RV E' data not included from those 20 studies in which no E velocity present.

9.8 CTDI velocities and PAP

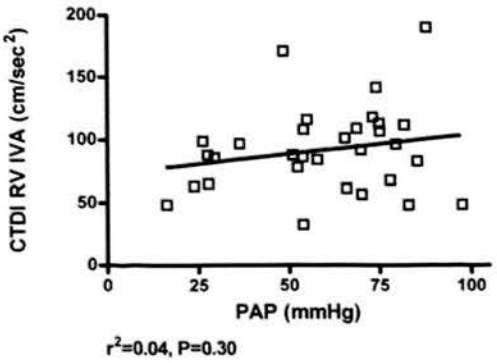
CTDI velocities were obtained in only 33 (71%) of the studies in which paired PAP data were also obtained. In seven studies CTDI data were not obtained because the studies were discontinued to allow ongoing clinical care. In the six other studies CTDI data were not collected because the decision to include CTDI (as a means of assessing RV function) was made after recruitment to the study had already commenced. All data presented refer to the 23 studies in which paired CTDI and PAP data were collected.

9.8.1 CTDI systolic velocities and accelerations

RV IVA, RV IVV and RV S were obtained as described in Chapter 2. Mean RV IVA was 88.1 (32.6) cm/sec^2, and did not significantly correlate with PAP (r^2=0.04, P=0.30, Figure 9.6, panel A). Mean RV IVV was 1.2 (0.6) cm/sec and also did not correlate with PAP (r^2=0.001, P=0.9, Figure 9.6, panel B). Mean RV S was 2.2 (1.0) cm/sec and did not correlate significantly with PAP (r^2=0.01, P=0.52, Figure 9.6, panel C).

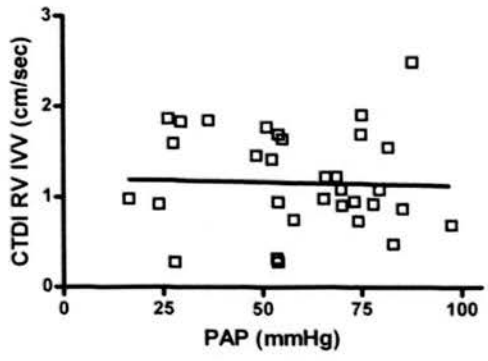
Figure 9.6: CTDI systolic parameters and PAP

A: RV IVA and PAP



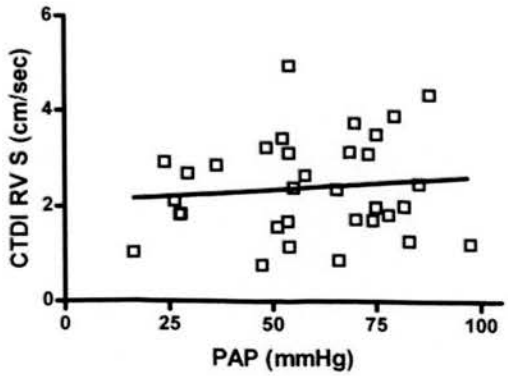
r^2=0.04, P=0.30

B: RV IVV and PAP



r^2=0.001, P=0.9

C: RV S and PAP

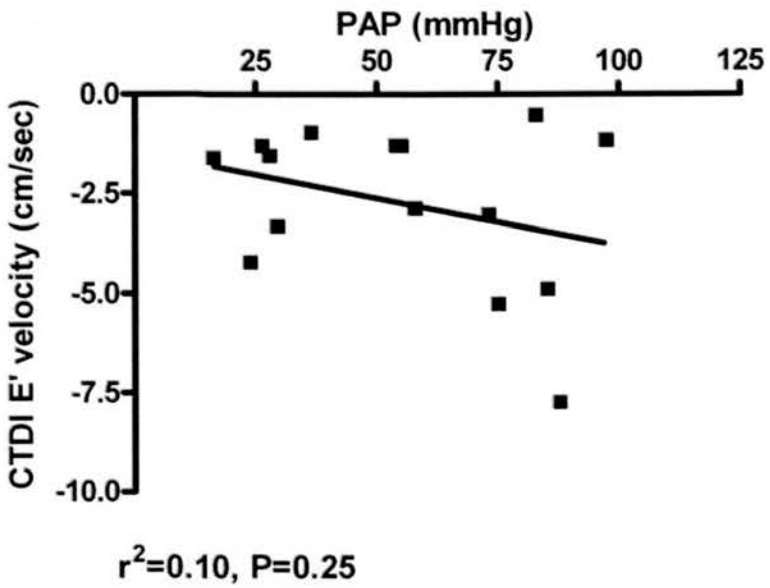


r^2=0.01, P=0.52

9.8.2 CTDI diastolic velocities and PAP

CTDI E' velocities were absent in 18 studies and these were not included in the investigation of the relationship between E' and PAP. Accordingly E' velocity was only present and measured in 15 studies. Mean CTDI E' velocity was -2.8 (2.0) cm/sec. CTDI E' velocity did not correlate with PAP; R^2=0.10, P=0.25. CTDI E' and PAP are plotted together in Figure 9.7. CTDI A' velocity was not measured as A' velocities were deemed to reflect atrial, and not ventricular function.

Figure 9.7: CTDI E' velocity and PAP



9.9 Discussion

This study investigated the relationship between PAP and RV function. The hypothesis tested was that, in infants with PHT, RV function is not linearly related to PAP. The clinical relevance of this study is in determining whether assessment of PAP pressure alone is sufficient to predict RV dysfunction in PHT, or whether direct assessment of RV function itself is required.

9.9.1 Measurement of PAP

For the purpose of this study PAP was quantified using the peak TR velocity and inserting this into a modified Bernoulli equation. This provided the peak pressure gradient between RV and RA (at end systole). The PAP was then calculated by adding right atrial pressure to the gradient. This technique has been previously described in infants and is widely used in clinical practice [96, 97].

In this study 65 paired measures of PAP and RV function were attempted. However, a TR jet was present, and a satisfactory Doppler obtained of it, in only 46 of these measures (70%). Skinner et al have previously reported the incidence of pansystolic TR as 53% in preterm infants and 27% in term infants [97]. Our findings confirm the finding that TR is not present in all infants, even when RV pressures are pathologically elevated. PAP may therefore not always be quantified by this method.

9.9.2 Relationship between PAP and measures of RV function

There was no linear correlation between PAP and RV function assessed using RV_{MPI} or tissue Doppler imaging (PWTDI or CTDI systolic and diastolic velocities).

One interpretation of this finding is that PAP does not correlate linearly with RV function, in support of the hypothesis being tested. It must be borne in mind, however, that the echocardiographic indices used may not be pure measures of RV function alone. RV_{MPI}, in particular, is load dependent and any change in this index may be the direct effects on change in load, independent of change in ventricular function. It is of note that the current study findings are in contrast to previous investigations of the relationship between RV_{MPI} and PAP in animal and paediatric studies. Suguira et al used hypoxia to induce PHT in a newborn piglet model, and demonstrated a significant correlation between RV_{MPI} and PAP (r^2=0.799, $p<0.05$) [73]. Dyer et al measured PAP in 12 children with idiopathic pulmonary hypertension and observed a significant positive correlation between RV_{MPI} and PAP (r^2=0.94, $P<0.001$) [75]. In light of these findings these authors had suggested that RVMPI might be used as a proxy measure of PAP. The current study findings, however, do not support the use of RV_{MPI} as a proxy measure of PAP in infants with PHT.

Tissue Doppler indices are potentially more robust, less load-dependent, measures of RV function than RV MPI. The absence of a relationship between PAP and any TDI measure (systolic and diastolic PWTDI and CTDI velocities) is arguably more convincing evidence of the genuine absence of a relationship between PAP and RV function. The absence of diastolic TDI E' velocities, in some studies, did however raise some difficulties in analysis. As discussed in chapters 7 and 8 the absence of early diastolic velocities in PHT was considered an important finding and indication of early diastolic dysfunction. Use of this data in statistical analysis was nevertheless

not straightforward. Ultimately, it was not considered valid to attribute a velocity of zero in these cases, and instead they were excluded from statistical analysis, which was therefore limited to those studies in which an E velocity could be identified and measured.

The same issue applied in the interpretation of tricuspid valve Doppler data, since E velocities were absent in the majority of these studies too. In the remaining infants in whom E velocities were present, a positive correlation with PAP was observed. This was interpreted cautiously, as not only was this data from only a small number of studies, but TV Doppler is a highly load dependent measure i.e. TV Dopplers cannot be considered to reflect RV function alone. This highlights the difficult of interpreting TV Doppler velocities and the limitations of this technique as a measure of RV function.

RVO was also observed to significantly correlate with PAP. Interestingly this correlation was positive, i.e. as PAP increased RVO increased, which is consistent with the finding in Chapter 5 that RVO tended to be higher in PHT infants than control infants. Can this be interpreted as indicating that RV myocardial function increases with PAP? RVO is determined by numerous factors, including preload, afterload, RV myocardial function and intra- and extra- cardiac shunts. On the basis of the current study, the correlation between RVO and PAP cannot therefore be exclusively attributed to a relationship between RV function and PAP.

One explanation for the increase in RVO with PAP, independent of any change in RV myocardial function, is that at higher PAP there is increased right-to-left shunting through the patent ductus arteriosus, and hence increased RVO. Supra-normal RVO due to massive right-to-left ductal shunts in infants with severe PHT has previously been observed in infants with Vein of Galen malformation [16]. These data illustrates the numerous factors which may affect RVO, and its unsuitability as a measure of myocardial function alone.

9.9.3 Significance of the absence of a relationship between PAP and RV function

The absence of a clear linear relationship between PAP and RV function demonstrated by this study (with the exclusion of TV Doppler and RVO data for the reasons discussed above) is significant to the clinical setting. On the basis of this conclusion RV function cannot be easily predicted from PAP. This emphasises the importance of assessing RV function directly in infants with PHT, and not simply PAP alone.

Why might RV function (systolic and diastolic) not closely correlate, in a linear manner, with PAP? Two possible explanations were considered:

1. RV systolic function is not inherently related in a linear fashion to afterload i.e. the ventricle does not have the ability to increase directly and proportionately its systolic (contractile) and diastolic (lusitropic) function in

response to increasing afterload. The non-linear response of the ventricle to changes in load has previously been well described as the bell-shaped Frank-Starling relationship between preload and contractile function.

2. As PAP increases in infants with PHT there are concomitant changes in other factors which may independently alter RV function in a non-linear manner. Such factors were not studied or controlled for in this study, and may have included exogenous therapy (nitric oxide, ventilation settings, inotrope dose), and metabolic disturbances such as hypoxia, hypercarbia or acidosis.

Future studies are necessary to investigate the relationship of RV function and PAP to test these hypotheses.

9.9.4 Limitations of this study

Limitations of the techniques used to measure RVO have been discussed above. Limitations related to the use of TR velocity to calculate PAP have also been highlighted. Specifically, error may have been introduced if the continuous wave Doppler beam was not positioned within the highest flow region of the regurgitation jet, or if the maximum velocity was not accurately measured from the resultant Doppler waveform. Furthermore, as with any Doppler velocity measurement, if the angle of incidence of the Doppler beam was greater than 15^{o} then the measured velocities may have been lower than true velocities. For these reasons care was taken to select only those 46 studies in which TR was present and a satisfactory

Doppler waveform obtained, from a minimal angle of insonation, and from which peak velocity could be clearly identified and measured.

Calculation of PAP required an estimation of right atrial pressure (RAP) of 5 mmHg in every study. RAP could not be measured invasively as this would have created unacceptable associated risks. It is probable that true RAP was higher than 5 mmHg, due to the combination of tricuspid regurgitation and/or increased venous return. Nevertheless, the relative contribution of RAP to the total calculated PAP is small, and unlikely to have significantly altered the results.

RV function measures (transtricuspid Doppler flow, RVO measurements, RV_{MPI} measurements and TDI data) were collected after collection of the TR Doppler. PAP and RV function measures were therefore not simultaneous, but separated temporally by up to 5-10 minutes. It is possible that either PAP and/or RV function may have changed during this time period, and that this may in turn have affected the results of the correlation analysis.

As PAP increases, in the clinical setting, other factors may change too which directly alter RV function, independently of PAP. Such factors have been discussed above, and include changes in oxygen and carbon dioxide partial pressures, acid base status, and changes in exogenous therapy e.g. ventilation mode and settings, inspired FIO_2, and inotrope dose. No attempt was made to control for these factors and examine in strict isolation the relationship between RV function and PAP. It is possible that in a controlled setting, where extraneous changes are minimised, RV function may

correlate linearly with PAP, as previously reported by Dyer et al and Suguira et al [73, 75]. The current study is more relevant to the clinical setting where these varied factors cannot be controlled for, and PAP is therefore not seen to correlate with RV function.

Each measure of RV function was not completed in every study. In three studies RVO and RV_{MPI} data was not collected, in nine studies PWTDI data was not completed and in 23 studies CTDI data was not completed, for the reasons discussed earlier. This highlighted the difficulties of performing a clinical study where collection of study data was frequently interrupted to allow ongoing clinical care. Finally, the absence of early diastolic velocities in TV Doppler, PWTDI and CTDI data created difficulties in data analysis. Ultimately, it was felt most valid to exclude those studies in which no early diastolic velocity was present from statistical analysis (as opposed to attributing a velocity of zero).

9.10 Conclusions

No significant correlation was observed between PAP and RV function, assessed using RVMPI, PWTDI and CTDI velocities. This supports the hypothesis that RV function does not correlate with PAP. An important clinical implication of this finding is that RV function cannot be predicted from PAP, and that RV function should be independently assessed.

PAP did correlate with transtricuspid E velocities but these are a highly load dependent measure and cannot be considered to reflect accurately myocardial function alone. A positive correlation between PAP and RVO was also observed, which was probably due to increased right-to-left ductal shunting in severe PHT. These findings, rather than disproving the tested hypothesis, highlight the major deficiencies of these measures as means of independently assessing RV myocardial function.

CHAPTER 10

DISCUSSION AND CONCLUSIONS

10.1 Introduction

This chapter considers whether the studies have answered the research questions posed at the beginning of this work, and what has been added to existing knowledge and theory.

There are three principle sections, which correspond to the original research questions;

1. Methods of measuring RV function in infants,
2. Mechanisms of RV dysfunction in PHT
3. The relationship of RV function and PAP

In each section the results of the current work are used to argue for the conclusions drawn. Limitations of the current work relating to methodology, study population and the echocardiographic techniques, have been discussed in detail in the preceeding results chapters. However, where relevant these are briefly discussed again here. As a consequence of the data presented new areas for future research are identified.

10.2 Methods of measuring RV function in infants

The first research question stated in this thesis was: *Which measures can be used to assess RV function in infants with PHT?*

The corresponding hypothesis was that: *RV function can be quantified in the clinical setting by non-invasive echocardiographic measures*

This was considered a relevant research question because RV function is an important consequence of pulmonary hypertension, and a determinant of disease severity, however in current clinical practice there are no accepted conventions for quantifying RV function in infants.

The current studies aimed to identify a feasible, quantitative technique for assessment of RV function in infants. A number of echocardiographic measures were investigated, chosen on their likely ability to meet the "ideal" criteria of being rapidly obtained, accurate, load-independent and allowing quantification of both diastolic and systolic function. These included the previously described techniques of tricuspid valve Doppler, RVO and RV_{MPI}, and the new technique of TDI. The study findings in relation to each of these techniques are now discussed.

10.2.1 Tricuspid Valve Doppler

Tricuspid valve Doppler is a proven technique for measuring diastolic ventricular filling in newborn infants [55], but is rarely performed routinely in infants in clinical practice. The current studies have confirmed the practicality of this technique, the principle strength of which was found to be its simplicity.

The problems of TV Doppler as a measure of RV diastolic function were evident on attempted interpretation of the study data. Atrio-ventricular Doppler flow velocities are highly load-dependent [26, 126]. Accordingly, it could not be known whether the observed difference in TV E velocity between control and PHT infants in the current studies was due to differences in loading conditions alone or real differences in RV diastolic function.

The interpretation of TV Doppler velocities in an individual infant was also complicated by physiological variation in velocities with post-natal age [67]. A further limitation of TV Doppler as a means of assessing RV function is that this technique provides no assessment of systolic function.

Taking into account the practical strengths but interpretative difficulties of TV Doppler, does this technique have any value for use in the clinical setting? Accepting that TV Doppler is a measure only of flow, and cannot distinguish the varied influences of load and RV function, it is proposed to use TV Doppler as a rapid "first-line" screening measure of RV diastolic haemodynamics in infants. The

technique could be used to detect abnormal diastolic filling patterns and identify those infants in whom further analysis of RV function is warranted.

This proposed use of TV Doppler in infants to "screen" RV function, is similar to the existing established use of mitral valve Doppler to assess for LV diastolic dysfunction in adults with ischaemic heart failure [27, 139].

10.2.2 Right Ventricular Output

RVO has been previously proposed as a measure of RV function, as well as providing a measure of systemic blood flow (cardiac output) [56, 61]. The studies conducted here however demonstrated both practical limitations of this technique and that RVO cannot be used to assess RV myocardial function in isolation.

Measurement of RVO was found to be time consuming requiring multiple echocardiographic measures and protracted post-acquisition analysis. Furthermore, each measurement was prone to error and the multiplication of these to calculate RVO had the potential to generate even greater error. Such error may have contributed to differences in "normal" RVO observed between current and prior studies [61, 65].

In addition to practical limitations, RVO failed to provide a measure of RV myocardial function alone. In the PHT group, RVO was not reduced compared to the control group even though RV dysfunction was present, as demonstrated by other

echocardiographic measures e.g. TDI. The maintenance of RVO in PHT was attributed to a compensatory increase in heart rate in this group. This emphasises that RVO is a global, composite measure of cardiac performance which is dependent not only on myocardial function but also on heart rate, preload and afterload.

In view of its global nature and practical limitations RVO is not an appropriate technique for assessing myocardial function in the clinical setting. Furthermore, the current studies have demonstrated that RVO is preserved even in the face of significant RV dysfunction. Whilst this probably demonstrates the ability of the newborn infant circulation to compensate acutely for myocardial dysfunction and maintain cardiac output, it also highlights that RVO is a poor measure for the clinician wishing to identify and treat RV dysfunction immediately.

The trend towards increased RVO in the PHT group, compared to controls, was hypothesised to be due to the presence of a right-to-left shunt through the patent ductus arteriosus in the majority of PHT infants. In these infants, RVO represents the sum of pulmonary blood flow and a proportion of systemic blood flow. Although this study was not specifically designed to assess the effects of the patent ductus on RV function in PHT, this is an area of current clinical interest which has not been previously studied and is therefore considered briefly here.

A number of authors have proposed that maintaining ductal patency in infants with severe PHT, using prostaglandin E1, may reduce afterload on the RV and provide a "blow-off" valve to prevent RV dilatation and dysfunction when pulmonary vascular

resistance exceeds systemic vascular resistance [17, 38]. This approach has been associated with improved outcome in the setting of PHT due to CDH [118]. However, no studies have previously examined the haemodynamic consequences for the RV of promoting right-to-left ductal shunting in PHT. The data obtained in this study was the first measurement of RVO in the presence of right-to-left shunting. That RVO was increased in some infants with PHT brings into question whether the presence of the patent ductus actually increased RV work and might worsen the extent of myocardial dysfunction. This finding raises an important area for future study; to determine whether the practice of maintaining ductal patency in infants with PHT benefits RV function by reducing afterload or compounds RV dysfunction by increasing RVO and therefore RV work.

10.2.3 RV_{MPI}

The MPI was developed as a practical, quantitative measure of ventricular performance which has the advantages of being independent of angle of insonation, assessing both systolic and diastolic function and providing a means of quantifying ventricular function [69, 70]. The studies conducted here confirmed the feasibility of performing RV_{MPI} in normal infants, and were in good agreement with prior reports [111, 134, 135]. The current studies also demonstrated, for the first time, the feasibility of performing RV_{MPI} in infants with PHT. The finding of elevated RV_{MPI} in infants with PHT, independent of age, was in agreement with previous studies in adults and children with PHT, and newborn piglet models of PHT [73, 75].

The problems of RV_{MPI} as a measure of RV function become apparent when trying to interpret the increase in RV_{MPI} in the PHT group. RV_{MPI} has been shown to be highly load-dependent in conductance catheter studies [76] and it is therefore unclear whether the observed increase in RV_{MPI} was due to a change in myocardial function, preload or afterload. Even if changes in RV_{MPI} directly reflect change in myocardial function (independent of changes in load), the "global" numerical index provides no indication of whether systolic or diastolic function, or indeed both, has changed.

In view of the load dependence and global nature of RV_{MPI}, like RVO it cannot be recommended as a specific measure of RV myocardial function. Nevertheless, given the practical ease of acquiring this index it may still have a role as a "screening" measure. For example, the detection of an elevated RV_{MPI} in an infant might be used as an indication to perform more detailed assessment of RV function. In addition RV_{MPI} may have a role in the serial scoring of combined RV function and loading conditions in infants with PHT.

10.2.4 Pulse Wave Tissue Doppler Imaging

Tissue Doppler imaging is a new echocardiographic technique which allows measurement of myocardial velocities and time intervals as indicators of systolic and diastolic myocardial function [77]. The current studies are the first reported use of TDI to assess RV function in infants with PHT or any other disease process. These studies were therefore very important as investigations of the feasibility of using TDI in this setting, and as a potential means of providing new understanding of the

mechanisms of RV dysfunction in PHT. Two forms of TDI were employed: pulse wave TDI (PWTDI) and colour TDI (CTDI).

The current studies confirmed the feasibility of PWTDI in normal infants and provided normative myocardial velocity data which was in good agreement with the two prior reports [94, 105]. The feasibility of PWTDI in infants with PHT was also confirmed for the first time. PWTDI data were obtained rapidly and without clinical deterioration in all infants in whom clinical circumstances allowed. PWTDI data analysis were successful in all but one infant with PHT, in whom artefact from HFOV prevented analysis. This was not encountered in other infants receiving HFOV and therefore this ventilation mode should not preclude use of PWTDI.

Systolic and diastolic myocardial PWTDI velocities (IVV, S, E' and A') were successfully obtained in both control and PHT infants. However, isovolumic acceleration (IVA) could not be accurately measured due to the combination of high infant heart rates and spectral broadening of the PWTDI trace. PWTDI IVA cannot therefore be recommended as a feasible measure of myocardial function in infants.

Interpretation of PWTDI myocardial velocities highlighted the major advantages of these measures of myocardial function over TV Doppler, RVO or RV_{MPI}. Firstly, systolic and diastolic function may be independently measured. Secondly, the early and late components of diastolic function can be distinguished, allowing assessment of early diastolic relaxation (a combination of active relaxation and passive compliance or stretch) and late diastolic function related to atrial contraction. No

other non-invasive measure of ventricular function allows such detailed analysis of function throughout the cardiac cycle.

The load-dependence of TDI velocities remains an area of controversy, which must be borne in mind in data interpretation. Existing studies would suggest that, outwith extremes of loading, systolic TDI velocities are relatively load-independent measures of myocardial function [49, 82]. TDI E' velocities demonstrate preload dependence in the normal heart, but not the failing heart [85, 86]. The velocities in the current study were considered to reflect more accurately true myocardial function than TV Doppler, RVO or RV_{MPI}. Future studies are required to investigate the true degree of load-dependence of TDI velocities in infants.

Unlike PWTDI velocities, the diastolic time interval IVRT (and its reciprocal measure, DMDT) cannot be recommended as measures of RV myocardial function, as these were difficult to measure in some PHT infants (due to post systolic positive velocities) and are known to be highly load-dependent [27].

The demonstration of PWTDI velocities as feasible measures of both systolic and diastolic RV function in infants may be one of the most important findings of this thesis. Can PWTDI be recommended as a practical clinical measure of RV function in all infants with PHT on the basis of the current findings? This study has confirmed that PWTDI is safe and practicable for use in infants with PHT, and has the potential to allow detection of myocardial dysfunction before other measures, such as RVO, are altered. Repeatability and reproduceability require assessment

before PWTDI can be recommended for routine clinical use. In addition, although there were statistically significant differences in myocardial PWTDI velocities between PHT and control groups, it is not clear whether these changes in velocity are of sufficient size to be clinically detectable in individual patients. Larger studies of PWTDI velocities in normal and PHT infants may provide an indication of the ranges of normal and abnormal velocities to aid clinical interpretation.

Another issue preventing routine use of PWTDI at present is that few neonatal echocardiographers have experience in use of PWTDI. Training would be required if this technique were to be put into routine practice. The availability of ultrasound machines capable of performing PWTDI may be another barrier to widespread introduction of this technique.

These studies have indicated the significant future potential of PWTDI as research tool in other neonatal diseases where myocardial dysfunction is suspected, including respiratory distress syndrome, preterm low cardiac output states, patent ductus arteriosus, sepsis and post-operative states [61, 102, 140]. Future studies could also employ PWTDI to investigate the effects of current and future therapies on RV function allowing future therapies to be developed which specifically target mechanisms of myocardial dysfunction.

10.2.5 Colour Tissue Doppler

Colour tissue Doppler is a more recently developed and sophisticated form of TDI, compared to PWTDI, which theoretically allows more rapid data acquisition and increased flexibility in the choice of size, distribution and number of myocardial regions for velocity analysis [77]. CTDI has never previously reported in infants. The current studies represented an important assessment of the feasibility of CTDI in infants.

The studies presented here demonstrated the ease with which CTDI "raw" data could be collected in infants, from a single echocardiographic window over a period of 3-5 beats. The problems of performing CTDI in infants became apparent during post-acquisition data analysis.

The combination of high infant heart rates and limited frame rate analysis led to insufficient sampling frequencies. The result was distorted CTDI waveforms in which some myocardial velocities could not be accurately identified and peak velocities were lower than "true" peaks. Subsequent inaccuracy in measuring the velocities was thought to have led to the wide variance of CTDI velocities. The shorter-lived IVV was particularly affected by this issue, making measurement of IVA inaccurate also. This was a disappointing finding, as one of the major potential advantages of CTDI over PWTDI was the theoretical ability to measure IVA without the problem of spectral broadening which had been encountered in PWTDI.

The issue of insufficient sampling frequencies is entirely due to the limitations of the current software and hardware used to acquire CTDI data. The combination of the Philips IE33 and QLab software used in these studies was one of the most advanced cardiac ultrasound packages currently available, yet still an optimal sampling frequency could not be achieved.

CTDI analysis was also time-consuming and considered vulnerable to measurement error due to potential mal-positioning of the Doppler sample outwith the myocardium.

In view of these problems, the CTDI data obtained in the control and PHT groups were interpreted with considerable caution. The control data, in theory, represents the first normative CTDI data in infants, but the wide variance of the individual velocities more likely reflects measurement error rather than true physiological variation within the control group. Comparison of CTDI velocities between control and PHT groups yielded inconsistent differences in systolic velocities, which were not in agreement with PWTDI data. The CTDI data was considered too inaccurate to provide meaningful insight into the changes in myocardial function in infants and is not included in the discussion of the mechanisms of RV dysfunction in PHT below.

On the basis of this work CTDI, in the form employed in this work, cannot be recommended as a measure of myocardial function in infants. Nevertheless, technical advances in the future may allow higher sampling frequencies, which could overcome the major current problems of CTDI use in infants.

10.2.6 Conclusions

No current echocardiographic index is a perfect measure of RV function. The issues of variable load-dependence, measurement error and limits of current technology all prevent direct and specific measurement of myocardial function in systole or diastole. All of the measures of RV function assessed in this work could be safely and feasibly performed in normal infants and infants with PHT. PWTDI was found to be the most useful measure of RV function in infants with PHT, providing quantitative assessment of both systolic and diastolic function. PWTDI is proposed as a means of assessing RV function in future research and clinical settings. TV Doppler is limited by its load dependence but allows rapid measurement of inflow as an assessment of function. RV_{MPI} and RVO are limited by their global nature and load dependence. CTDI is a promising technique but the limitations of current technology mean that it cannot be recommended for current use in infants.

10.3 Mechanisms of RV dysfunction in pulmonary hypertension

The second research question posed at the outset of this work was: *What are the mechanisms of RV dysfunction in infants with PHT?*

The related hypothesis was that "*RV dysfunction in PHT is characterised by systolic and diastolic dysfunction*".

This was considered an important research question as the mechanisms of RV dysfunction in infants with PHT have not been previously studied and better understanding of these may allow more informed therapeutic decisions.

The mechanisms of RV dysfunction in PHT were investigated by comparison of RV function between control and PHT infant groups. For the purposes of this work "mechanisms" of RV dysfunction refers to whether changes occurred in systolic and/or diastolic function in the RV. It was beyond the scope of this study to investigate changes in the RV at a morphological, cellular or molecular level.

Although a number of different echocardiographic measures were used to assess RV function in this work, only PWTDI and TV Doppler data were used to draw conclusions on the mechanisms of RV dysfunction in PHT. PWTDI data was considered the most informative as this provided assessment of both systolic and diastolic function. TV Doppler data also provided an indication of diastolic (but not

systolic) RV function in PHT, but was interpreted with caution in view of the direct load dependence of this measure. RVO, RVMPI and CTDI data were not considered useful in determining the mechanisms of RV dysfunction in PHT: RVO and RV_{MPI} are global, load-dependent measures of RV function which provided no indication of the relative changes in systolic and diastolic function. CTDI data was considered inaccurate due to measurement error.

10.3.1 Systolic RV function in infants with PHT

The PWTDI data demonstrated a significant reduction in systolic myocardial velocities during both isovolumic contraction (IVV) and ejection (S). PWTDI systolic velocities have previously been demonstrated to correlate well with gold standard conductance catheter measures of systolic function. The reductions in IVV and S velocities in the PHT group were therefore considered to reflect genuine impairment of systolic function during both early isovolumic contraction and later systolic ejection phases respectively.

This study represented the first direct demonstration of systolic dysfunction in this setting. Assessment of systolic function in PHT has previously been limited to animal models and adult studies. In animal models, the initial response of the RV to acute increases in afterload was an increase in RV systolic function [100, 141, 142] – attributed to a combination of the Frank-Starling mechanism and early compensatory RV hypertrophy. Chronic exposure to elevated afterload, however, leads to systolic dysfunction, as has been demonstrated in adults using TDI techniques [92, 93]. The current findings have demonstrated the same impaired systolic function in infants.

That no increase in RV systolic function was observed in the infant PHT group suggests that the infants studied had already undergone the "pivotal divergence" from initial compensated RV function with increased systolic function to a chronic failing RV with impaired systolic function [35].

10.3.2 Diastolic RV function in infants with PHT

Diastolic function has been rarely considered in infant disease states, principally due to the absence of acceptable measures. Use of PWTDI in the current work allowed the first direct assessment of diastolic myocardial function in infants with PHT. The finding of reduced early diastolic myocardial velocities (E') was considered to suggest impaired early diastolic function. This was one of the most striking findings of the current work.

The data presented here are in agreement with prior animal and adult studies. In both lamb and dog models of chronic PHT, RV diastolic dysfunction has been demonstrated using "gold-standard" conductance catheter techniques [90, 100]. In adults with primary PHT, TDI RV function has been assessed using the same TDI techniques employed here in infants [92, 93]. In these adults, just as in the PHT infants, E' velocities were significantly reduced indicating early diastolic dysfunction. Kjaergaard et al, also using TDI, have demonstrated the same impairment of early diastolic function in the RV in adults with acute onset, hypoxia-induced PHT [74].

Why is early diastolic function impaired in infants with PHT? Early diastolic function is a combination of active myocardial relaxation and passive chamber stiffness (or compliance) and consists of an initial isovolumic phase (IVRT) followed by early ventricular filling, corresponding to the PWTDI E' wave [26]. One possible clue as to the cause of impaired active relaxation, in the infants with PHT, was the presence of abnormal post-systolic positive myocardial velocities in the PWTDI data from these infants. It is hypothesised that these abnormal "post-systolic contractions" may have represented either abnormal contraction of the failing ventricle after pulmonary valve closure, or re-organisation of the RV morphology between systole and diastole. Whichever the explanation, this period of post systolic contraction may have prevented normal early active diastolic relaxation and ventricular filling. This represents an area for future study, both to identify the aetiology of the post-systolic contraction and the effects on early active myocardial relaxation.

The passive component of early diastolic function (i.e. RV compliance) might also have been affected in infants with PHT. In support of this, Leewenburgh et al have previously demonstrated impaired chamber stiffness (using catheter techniques) in the hypertrophied RV of lambs following pulmonary artery banding [90]. However, the echocardiographic techniques used in the current study did not allow direct assessment of chamber compliance.

10.3.3 Factors responsible for the observed changes in RV function in PHT

Increased afterload on the RV is likely to have been a major contributing factor to the development of systolic and diastolic dysfunction in the PHT group. However, other differences between the two study groups may also have contributed to the observed changes in RV function in the PHT group.

Infants in the PHT group were variably receiving a number of therapies which may have altered myocardial function either directly or by modulation of afterload. These included mechanical ventilation, inhaled nitric oxide, oral sildenafil, prostagladin E1 and muscle relaxation. In addition, 50% of the infants in the PHT group were receiving an inotrope (either dopamine, dobutamine or milrinone). The effects of these therapies on infant RV myocardial function have not been extensively investigated and are poorly understood despite their frequent use in the clinical setting. Metabolic factors, genetic factors and changes in ventricular morphology may also have contributed to altered RV function in the PHT group [143].

One major limitation of the current studies was the inability to control for, or investigate, the individual influences of these myriad factors on RV function. Further studies are required to determine the specific effects of these. On the basis of the data presented here it can only be concluded that RV systolic and diastolic function are impaired in infants with PHT, but not that PHT *per se* was the sole factor responsible for this.

10.3.4 Implications of study findings for therapies in infants with PHT

The findings of systolic and diastolic RV dysfunction in infants with PHT have important clinical implications for therapeutic choices in this setting.

Treatment of RV dysfunction in infants with PHT currently focuses on attempting to reduce afterload and directly improve RV function. The cathecholamine analogues dopamine and dobutamine are, historically, the most widely used cardiotropic agents in newborn infants, and are chosen for their "inotropic" actions i.e. their ability to increase systolic performance, or contractility [140]. On the basis of the current study findings, however, an ideal cardiotropic agent for use in infants with PHT would improve both systolic *and* diastolic (lusitropic) function.

One promising candidate agent is the phosphodiesterase inhibitor milrinone which has been demonstrated to improve both RV systolic and diastolic function in a canine model of PHT [100]. Studies into the use of milrinone in infants with PHT have been limited to case reports of the drug's successful use in combination with nitric oxide, to treat preterm infants with severe persistent pulmonary hypertension [144].

The current work provides a mechanistic rationale for the use of milrinone, or related agents, in infants with PHT. Future study is necessary in the form of a randomised controlled trial to investigate the potential benefits of milrinone in improving RV systolic and diastolic function, and reducing afterload, in infants with PHT.

10.3.5 Conclusions

RV dysfunction in infants with PHT is characterised by impaired systolic and early diastolic function. Therapies aimed at directly treating RV function in PHT should have both inotropic and lusitropic actions.

10.4 Relationship between RV function and pulmonary artery pressure

The third research question investigated in this thesis was: *What is the relationship between RV function and pulmonary artery pressure in infants with pulmonary hypertension?*

The corresponding testable hypothesis was: *that RV function does not correlate linearly with PAP.*

10.4.1 RV function measures and pulmonary artery pressure

This is an important question to address. If RV function is variable in PHT then measurement of PAP alone does not allow prediction of RV function, or measurement of illness severity. Conversely, if the RV function and PAP are linearly related then measures of RV function might be used as proxy measures of PAP, for example in those circumstances when a TR jet is absent and PAP cannot be directly measured.

The relationship between RV function and PAP was investigated, by repeated paired measurement of PAP and each of the measures of RV function (TV Doppler, RVO, RV_{MPI}, PWTDI and CTDI). There was poor linear correlation between measures of RV function and PAP. This was true even for those measures of RV function which are relatively load-independent (i.e. PWTDI and CTDI).

The absence of a linear relationship between RV function and PAP may indicate that RV myocardial function is not inherently linearly related to afterload, and cannot proportionately increase as PAP increases. Indeed, the non-linear response of the ventricle to changes in load is recognised in the bell-shaped Frank-Starling relationship.

Additionally, failure to demonstrate a linear relationship may have been due to the presence of other variable factors in the PHT group which modulated RV function. It is hypothesised that alterations in therapies (ventilation, inhaled NO, inotropes) and in metabolic condition, accompanying changes in PAP, altered the relationship between RV function and PAP. It is of note that, in studies in piglets and children where such variations in therapy were minimised, or absent, linear relationships between RV function and PAP were observed [73, 75, 101].

RV morphology and gene expression may also change in response to chronic pressure overload [143]. This in turn would conceivably lead to variable RV function at any given PAP, and the absence of a linear relationship between the two. Future studies investigating gene expression in the RV in PHT represent an interesting avenue of further research, and a potential area for development of new therapies.

The current work was not designed to determine the individual influences of these varied factors on RV function. Instead, in demonstrating the absence of a linear

relationship between RV function and PAP in the clinical setting, it has indicated the importance of directly assessing RV function in infants with PHT to determine the severity of RV dysfunction.

Finally, the failure to establish a linear relationship between RV function and PAP may have been due to experimental error. As discussed in Chapter 2, the use of tricuspid valve Doppler to measure PAP is dependent on an assumption of right atrial pressure, and on accurate measurement of peak tricuspid regurgitation jet velocity. Error in either of these variables may have affected PAP calculation.

10.4.2 Conclusions

This study has demonstrated that in infants with PHT RV function is not linearly related to PAP. This new finding has significant implications for the clinical assessment of infants with PHT. PAP cannot be used as a proxy measure of RV function and illness severity. Instead, RV function should be directly assessed in infants with PHT.

10.5 Overall Conclusions

The studies presented in this thesis have important implications for the assessment and management of infants with PHT. The demonstration that RV function is not linearly related to PAP emphasised the importance of measuring RV function directly. Such measures of RV function were investigated. TV Doppler and RVMPI allow rapid assessment of diastolic and global RV function respectively. This work

also included the first investigation of PWTDI in a newborn disease state and demonstrated the powerful potential of this technique to quantify both systolic and diastolic function. The subsequent demonstration of both systolic and diastolic RV dysfunction suggests a potential future role for new agents with both inotropic and lusitropic actions, and a possibility of improved outcome in this group of infants.

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APPENDICES

Appendix 1 : RV function data in congenital diaphragmatic hernia subgroup

	Control Group (n=28)	CDH subgroup (n=11*)	P
TV Doppler			
E (cm/sec)	0.52 (0.13)	0.35 (0.13)*	0.04
A (cm/sec)	0.55 (0.10)	0.63 (0.18)	0.34
RVO (mLs/kg/min)	273 (81)	258 (96)	0.44
RV_{MPI}	0.24 (0.09)	0.58 (0.18)	<0.0001
PWTDI			
IVV	6.6 (1.1)	5.2 (1.5)	0.007
S	9.2 (1.9)	6.3 (1.3)	0.0001
E'	-8.6 (2.0)	-3.6 (1.8)*	0.0006
A'	-10.1 (2.5)	-10.8 (4.3)	0.68
CTDI			
IVA	68 (30)	95 (39)	0.04
IVV	1.3(0.6)	1.4 (0.6)	0.54
S	2.6 (0.8)	1.9 (0.6)	0.03
E'	-3.3 (1.0)	-1.42 (0.4)*	0.007
A'	-3.4 (1.7)	-3.8 (1.9)	0.6

All data mean (SD).

* TV E, PWTDI E' and CTDI E' velocities were present in only four infants in PHT group. These velocities were absent in the remaining seven infants in PHT group, and were not included in statistical analysis.

Appendix 2: Published papers

Appendix 2.1:

Patel, N., Mills, J. F. and Cheung, M. M. H. '*Use of the myocardial performance index to assess right ventricular function in infants with pulmonary hypertension*', Pediatric Cardiology 2009; vol.30(2), pp.133-137

Summary

Objective

To measure and compare right ventricular (RV) function in normal infants and infants with pulmonary hypertension (PHT) using the myocardial performance index (RV_{MPI}) and to investigate the relationship between RV function and pulmonary artery pressure.

Design

A case control study in which RV_{MPI} was measured in 16 infants with PHT (of whom nine had congenital diaphragmatic hernia) and 28 normal control infants. Forty-three paired measures of RV_{MPI} and pulmonary artery pressure (estimated from tricuspid regurgitation jet velocity) were made in the PHT infants to allow investigation of the relationship between RV_{MPI} and pulmonary artery pressure.

Results

RV_{MPI} in control infants was 0.24 ± 0.09 (mean $\pm$ SD). RV_{MPI} was significantly elevated in the PHT group (0.55 ± 0.17, $P<0.0001$) including a subgroup of infants with PHT secondary to congenital diaphragmatic hernia (0.58 ± 0.18, $P<0.0001$).

There was poor correlation between RV_{MPI} and pulmonary artery pressure in the infants with PHT ($R^2 = 0.05$, P=0.17).

Conclusions

RV_{MPI} allows quantification of right ventricular function in infants and detection of RV dysfunction in PHT. RV_{MPI} was not linearly related to pulmonary artery pressure. Use of RV_{MPI} in the clinical setting must take into account the global and load-dependent nature of this measure.

Article proper:

Introduction

Pulmonary hypertension (PHT) is a cause of significant morbidity and mortality in the newborn infant (7). Pulmonary hypertension may be idiopathic or secondary to conditions such as congenital diaphragmatic hernia (CDH), meconium aspiration, pulmonary hypoplasia and sepsis (14).

In infants with PHT the ability of the right ventricle (RV) to function under increased afterload is an important determinant of illness severity. RV dilatation and dysfunction may in turn lead to impaired LV function due to ventricular independence, leading to poor cardiac output and worsening cardio-respiratory failure (4). Assessment of RV function is therefore central to management of infants with PHT, but has been complicated by the absence of a suitable clinical technique. Qualitative assessment of function by 2-dimensional echocardiography is the most widely used technique in current practice, but is subjective and has high inter-

observer variability (1). Furthermore, the complex geometry of the RV makes it unsuited to geometric measures of ventricular function such as ejection fraction and fractional shortening (2).

A reliable, quantitative measure of RV function would be useful in clinical practice to accurately assess function in PHT, monitor response to therapies, and predict outcome. The myocardial performance index (MPI), or Tei index, is a quantitative echocardiographic-derived measure of global (diastolic and systolic) ventricular function, which is non-invasive, simple to calculate and reproducible (17, 19). Right ventricular MPI (RV_{MPI}) has previously been used to quantify RV function in adults and children with PHT, where a linear relationship between RV_{MPI} and pulmonary artery pressure was reported (5, 23). RV_{MPI} has previously been measured in normal newborn infants and fetuses (3, 10, 12, 13), but not in infants with PHT and potential RV dysfunction.

The aims of this study were firstly to measure RV_{MPI} in a group of normal newborn infants and in a group of infants with PHT, including a subgroup of infants with congenital diaphragmatic hernia, and secondly to investigate the relationship between RV_{MPI} and pulmonary artery pressure.

Methods

This study was conducted in the Neonatal Unit of the Royal Children's Hospital, Melbourne, Australia between May 2006 and February 2007. Approval was granted by the local Ethics Committee.

Control Group

Control infants were recruited from the inpatient population. They had normal cardiovascular structure and function, were being treated for unrelated illness, were self-ventilating in air and were not receiving any cardiac medication. There was no clinical or echocardiographic evidence of pulmonary hypertension in this group. Single echocardiograms were performed in each subject to measure RV_{MPI} for comparison with the PHT group.

PHT group

Infants with PHT were identified on admission on the basis of clinical suspicion or previous echocardiographic findings. Infants were eligible for inclusion if they had pulmonary pressures equal to or greater than half systemic pressure, when estimated from tricuspid regurgitation jet velocity (see below) or if they had significant right to left shunting through a patent ductus. Infants were excluded if they had structural heart disease, other than a patent ductus. Infants received routine clinical management, including respiratory and cardiovascular support as deemed necessary by the attending medical team.

Initial echocardiograms were performed on PHT infants at the time of admission. RV_{MPI} was calculated from these studies to allow comparison with RV_{MPI} in controls. In order to investigate the relationship between RV_{MPI} and pulmonary artery pressure, repeated echocardiograms were performed in the PHT infants, during their admission, from which paired RV_{MPI} and peak TR velocity could be obtained.

Studies repeated in the same infant were performed between one and four weeks apart.

Echocardiographic measurements

Measurements were made using a Philips™ IE 33 (Philips Medical, Andover, MA, USA) using a 12 MHz or 8 MHz probe. Tricuspid valve Doppler flow was recorded from an apical four chamber view using pulse wave Doppler, with the sample volume positioned at the valve tips. Pulmonary valve Doppler flow was recorded from a left parasternal view with the sample volume placed at the level of the pulmonary valve tips.

Pulmonary artery pressure was calculated using a modified Bernoulli equation from the tricuspid regurgitation (TR) jet velocity obtained from the apical 4 chamber view using continuous wave Doppler (24). The pressure gradient calculated in this way represents the pressure difference between right atrial and right ventricular pressures. Pulmonary artery pressure was estimated by adding 5 mmHg (an estimation of right atrial pressure) to this gradient. In the absence of an adequate TR jet, pulmonary pressures were estimated by assessment of the pattern of shunting through the patent ductus arteriosus, using pulse-wave Doppler from a high left parasternal view (11).

MPI calculation

RV_{MPI} is the sum of RV isovolumic contraction time (ICT) and isovolumic relaxation time (IRT) divided by the RV ejection time. RV_{MPI} was calculated in practice from tricuspid valve inflow and pulmonary valve outflow Dopplers, as shown in Figure 1.

The time period from cessation to beginning of tricuspid inflow (a) and the right ventricular ejection time (b) were measured. RV_{MPI} is equal to (a-b)/b. Doppler-derived time intervals were measured offline from stored data and averaged over five consecutive cardiac cycles. Care was taken to select paired pulmonary and tricuspid Doppler flows between which R-R intervals varied by less than five beats per minute.

Statistical analysis

Demographic data (weight, gestation and age) were compared between control and PHT groups using unpaired t-tests. All RV_{MPI} data are expressed as mean and SD. RV_{MPI} for the PHT group, and CDH subgroup, were compared with control RV_{MPI} using Mann-Whitney tests. The relationship between RV_{MPI} and pulmonary artery pressure was investigated by linear regression analysis. Subgroup analysis of infants with PHT and CDH was performed. This data was of particular interest within our institution which has a specialist interest in the management of infants with CDH.

Results

Twenty-eight infants were recruited to the control group and 16 to the PHT group. Causes of PHT were CDH (eleven infants), PPHN (two infants), meconium aspiration (one infant), vein of Galen malformation (one infant) and congenital cystic adenomatoid malformation (one infant). Demographic details are provided in Table 1. There were no significant differences between the groups based on weight, gestation or age.

RV_{MPI} was significantly elevated in the PHT group compared to the control group (0.55±0.17 vs. 0.24±0.09 [P<0.0001] respectively; Table 2 and Figure 2). Sub-group analysis of the eleven infants with CDH demonstrated a significant elevation in RV_{MPI} this group alone (0.58±0.18), compared with the control infants (P<0.0001).

The relationship between RV_{MPI} and pulmonary artery pressure was also investigated. Paired measures of RV_{MPI} and were obtained on 43 separate occasions in the fourteen infants in the PHT group. There was poor correlation between these variables ($R^2 = 0.05$; P=0.17; Figure 3).

Discussion

This study has demonstrated a significant elevation in RV_{MPI} in infants with PHT compared to normal controls. There was, however, no correlation between RV_{MPI} and pulmonary artery pressure.

The MPI is an echocardiographic measure of ventricular function which is derived from Doppler flows across the ventricular inflow and outflow valves (17). MPI has been validated against invasive measures of cardiac function; it shows good reproducibility, heart rate independence and because it is not dependent on angle of insonation it can be measured rapidly from limited echocardiographic views (6, 20). RV_{MPI} has previously been used to assess RV function in adults, children and normal newborn infants (12, 18, 21). Our finding of an RV_{MPI} of 0.24 ± 0.09 in normal control infants is in good agreement with previous reports in normal newborn infants (3, 13).

RV_{MPI} has previously been measured in adults and children with primary PHT and by Sugiura et al in a newborn piglet model of hypoxia-induced PHT (5, 16, 23). These studies demonstrated a significant elevation in RV_{MPI} in PHT and suggested that RV_{MPI} may be used clinically to quantify RV dysfunction. Our study has demonstrated elevated RV_{MPI} in newborn infants with PHT of varied cause, including congenital diaphragmatic hernia.

RV_{MPI} has been proposed as a prognostic marker of outcome in adult disease states including PHT, acute myocardial infarction and cardiac failure (7, 8, 23). Tei et al demonstrated that in adults RV_{MPI} independently predicted cardiac death or need for transplantation in PHT (23). RV_{MPI} has also been employed as a means of assessing RV function in response to pulmonary vasodilator therapies in adult PHT (15). Further studies are now required to investigate the role of RV_{MPI} in predicting outcome and assessing therapeutic response in infants with PHT.

Previous studies have shown good correlation between RV_{MPI} and pulmonary artery pressure and have suggested that RV_{MPI} may be used as a proxy measure of the latter (5, 16). Our study did not support this finding. RV_{MPI} correlated poorly with pulmonary artery pressure calculated from tricuspid regurgitation jet velocity. One interpretation of this finding is that RV function is itself not linearly correlated with pulmonary artery pressure, a finding that is well recognised clinically (4).

Another possible explanation of the poor correlation between RV_{MPI} and pulmonary artery pressure is that RV_{MPI} was being altered by other factors. The PHT infants in this study were receiving standard clinical management as deemed necessary including cardio-respiratory support. It is possible that these therapies may have influenced RV_{MPI}, either by directly altering myocardial function or loading conditions. We have previously demonstrated that MPI is significantly directly affected by changes in afterload or preload without alteration of myocardial function (22). This load-dependence means that RV_{MPI} cannot be considered a pure measure of myocardial function.

Another limitation of RV_{MPI} is that it is a global measure of ventricular function which cannot be used to distinguish between systolic and diastolic dysfunction and therefore cannot inform the clinician's choice of inotropic or lusitropic therapy. Furthermore, because of the way the index is calculated a decrease in systolic function may be masked by an increase in diastolic function and vice versa.

Limitations of this study

We calculated MPI from tricuspid valve and pulmonary valve Doppler flows which were obtained consecutively over a number of minutes. However, physiological changes in heart-rate between these two recordings can introduce inaccuracy (10). We studied only a small group of infants, with heterogeneous diagnoses. Age at inclusion in the study also varied widely which may impact on our results since RV_{MPI} appears to fall within the first days of life in normal infant (1). Any inaccuracy in the estimate of pulmonary artery pressure from tricuspid regurgitation

jets will invalidate the analysis of the relationship of pulmonary pressure and RV_{MPI}. Care was taken to include only optimal TR Doppler flows, from which peak velocity could be clearly measured, but this led to exclusion of some data which may have biased the results. We assumed a right atrial pressure of 5 mmHg in all calculations of pulmonary artery pressure, but true atrial pressures may have been significantly higher. However, even if true atrial pressures were higher the relative contribution to the calculated pulmonary artery pressures would have been small and unlikely to significantly alter the results obtained.

Conclusion

RV_{MPI} can be easily measured in newborn infants and is elevated in those with pulmonary hypertension. However, use of RV_{MPI} in this clinical setting should take into account the global nature and load dependency of this measure.

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Tables

Table 1: Demographic details of control and pulmonary hypertensive groups (Mean (range))

	Controls (n=28)	PHT (n=16)	P (Controls vs. PHT group)
Male Sex	17	8	-
Weight (kg)	3.31 (2.1-4.9)	3.40 (2.7-4.7)	0.34
Gestation (weeks)	38 (28–42)	39 (36-41)	0.08
Corrected gestational age (weeks)	40 (36-48)	41 (36-44)	0.51

Table 2: RV_{MPI} in control group, pulmonary hypertension (PHT) group and congenital diaphragmatic hernia subgroup (CDH)

Group	RV_{MPI}
Control infants	0.24 (0.09)
All PHT infants	0.55 (0.17)*
CDH subgroup	0.58 (0.18)*

*P<0.0001 compared to control group

Figures

Figure 1: Calculation of RV_{MPI}

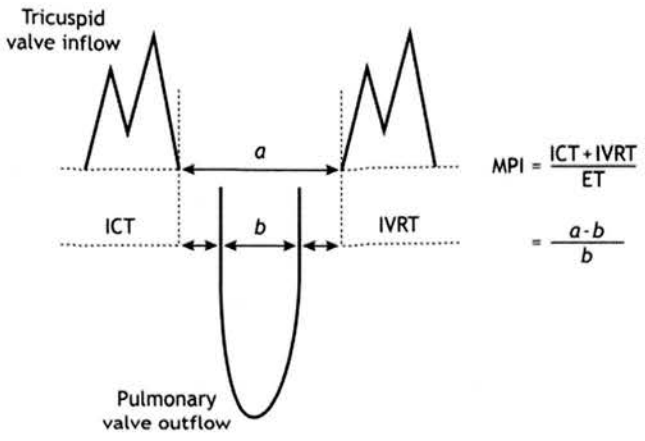
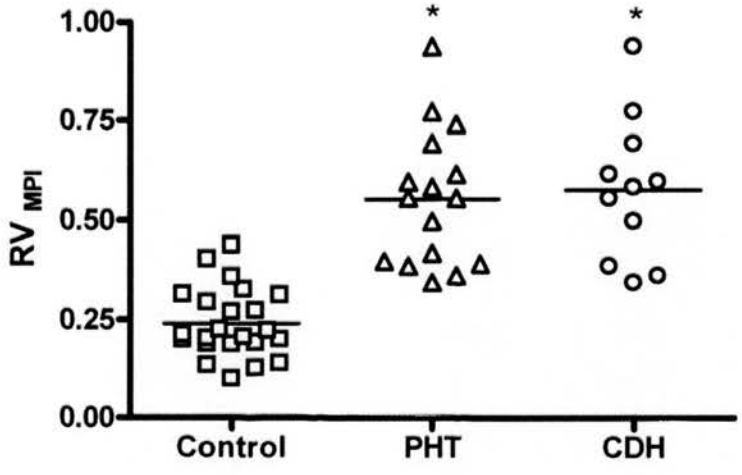
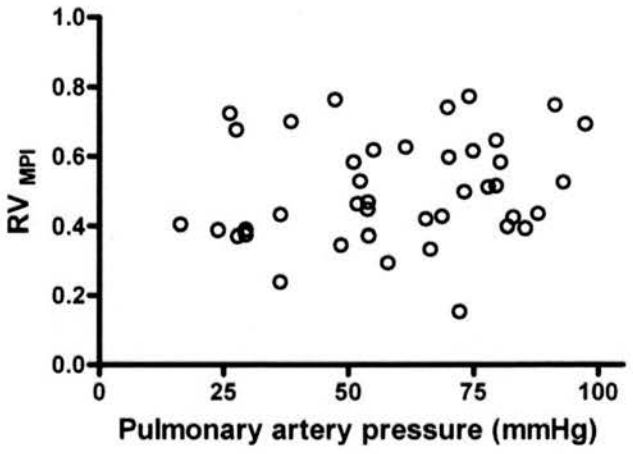


Figure 2:
RV_{MPI} in Control, Pulmonary Hypertension and CDH infants



*P<0.0001 compared to Control Group

Figure 3
RV_{MPI} vs. pulmonary artery pressure



Correlation between RV_{MPI} and pulmonary artery pressure was poor; R^2=0.05, P=0.17

Appendix 2.2:

Patel, N., Mills, J. F. and Cheung, M. M. H. *Assessment of right ventricular function using tissue Doppler imaging in infants with pulmonary hypertension.* Accepted for publication in Neonatology

ABSTRACT

Background

In infants with pulmonary hypertension, right ventricular (RV) function may be altered and contribute to disease severity. Tissue Doppler imaging (TDI) is a new echocardiographic modality which directly measures myocardial velocities and may allow quantative assessment of systolic and diastolic ventricular function in infants.

Objective

To measure and compare RV myocardial velocities in infants with pulmonary hypertension and in normal control infants, using TDI.

Methods

This was a prospective, case control study. Twenty-eight control infants and 15 infants with pulmonary hypertension (PHT), of whom 11 had congenital diaphragmatic hernia (CDH) were recruited. Tissue Doppler imaging (TDI) was used to obtain systolic and diastolic myocardial velocities in the RV and interventricular septum in all infants.

Results

There were significant reductions in systolic isovolumic contraction velocity (IVV; 5.3 vs. 6.6 cm/sec) and systolic ejection velocity (S; 6.6 vs. 9.2 cm/sec) in the PHT group compared to the control group. Early diastolic myocardial velocity, E', was also significantly reduced in the RV in the PHT infants compared to controls (-4.3 vs. 8.6 cm/sec). The same significant reductions in systolic and early diastolic TDI velocities were observed in the sub-group of CDH infants alone.

Conclusions

TDI permits non-invasive assessment of RV myocardial velocities in infants. Reduced systolic and diastolic velocities in PHT may represent impaired systolic contraction and early diastolic relaxation. Therapies which target inotropic and lusitropic function may be appropriate in infants with PHT and RV dysfunction. The load-dependency of TDI measures in infants, and the effects of specific therapies on RV function in PHT requires further investigation.

Article proper

INTRODUCTION

Pulmonary hypertension (PHT) is a cause of significant morbidity and mortality in the neonatal population, including in infants with congenital diaphragmatic hernia, meconium aspiration, sepsis and pulmonary hypoplasia [2]. Irrespective of the underlying cause of PHT, a principal consequence and a major determinant of disease severity is right ventricular (RV) dysfunction due to increased afterload [35]. RV function, or dysfunction, in infants with PHT may be highly variable and may not be predicted by measurement of pulmonary artery pressure alone [145]. A missing element in the clinical management of infants with PHT has been the absence of objective, quantitative measures of RV function and an understanding of the mechanisms of RV dysfunction on which to base therapeutic decisions.

Tissue Doppler Imaging (TDI) is a new echocardiographic technique which directly measures the velocities of myocardial motion during systole and diastole. TDI myocardial velocities have been validated as measures of myocardial function against gold-standard conductance catheter measures [77]. TDI has been employed to assess RV function in animal and adult studies [91-93, 146] but its application in newborn infants has been limited, thus far, to normal infants [94]. TDI may be a useful non-invasive means of assessing RV function in infants with PHT, to elucidate mechanisms of RV dysfunction and guide therapy.

AIMS

The aim of this study was to measure RV myocardial velocities in infants with pulmonary hypertension and in normal control infants, using TDI. The hypotheses tested were, that in newborn infants:

- RV myocardial systolic and diastolic velocities can be measured using tissue Doppler imaging
- Both systolic and diastolic RV function are altered in pulmonary hypertension

MATERIALS AND METHODS

Study Population

This prospective case-control study was conducted in the Neonatal Unit (NNU) of the Royal Children's Hospital, Melbourne. Approval for the study was granted by the local institutional Ethics Committee. A Pulmonary Hypertension (PHT) group was prospectively recruited consisting of consecutive admissions with echocardiographic evidence of pulmonary artery pressures equal to, or greater than, two-thirds peak systemic pressure. Infants were excluded if they had structural heart disease evident on echocardiogram.

A control group was also recruited prospectively who had no echocardiographic evidence of pulmonary hypertension, and no structural or functional cardiac disease. Control infants were a convenience sample, inpatient in the NNU with unrelated non-cardiac diagnoses during the same period the PHT group.

Clinical data

The gestation, age, corrected age, sex, weight and diagnoses of the subjects were recorded. Systemic blood pressure, for estimation of pulmonary artery pressure in the PHT group, was recorded from the bedside monitor (Philips Intellivue Patient Monitor MP 80/90, Philips Medical Systems, Bothwell, WA) obtained via an intra-arterial catheter or cuff sphygmomanometer. Heart rate was recorded from a three-lead ECG recorded simultaneously with all echocardiographic data. Current therapies were recorded, including; invasive ventilation, inhaled nitric oxide, and use of inotropes.

Echocardiographic data

In each subject, an echocardiogram was performed to assess RV function using TDI and conventional tricuspid valve Doppler inflows. All echocardiograms were performed using a Philips IE33 (Philips Medical Systems Bothwell, WA) with an 8 Mhz probe, by a single operator (NP), and digitally recorded to DVD for offline analysis. Doppler data was obtained from a minimal angle of insonation (<15°). Each echocardiographic parameter was measured over five consecutive cardiac cycles and the mean was calculated. All echocardiographic data was collected and analysed by a single observer (NP). Control group data was anonymised. PHT group data was archived for ongoing clinical purposes and was therefore not anonymised. Accordingly, the observer was not blinded to study group status during data analysis.

Tissue Doppler Imaging

Pulse wave Tissue Doppler Imaging (PWTDI) was used to measure RV myocardial velocities. PWTDI data were obtained using an apical four chamber view from the lateral tricuspid annulus (RV) and basal interventricular septum, as previously described [94]. A Doppler sample volume of two millimetres was used. Figure 1 is a representative trace of PWTDI velocities during one cardiac cycle. Systolic velocities measured were peak isovolumic systolic velocity (IVV) and peak ejection systolic velocity (S), corresponding to early systolic isovolumic contraction and later systolic ejection phases [82]. Diastolic velocities measured were the early diastolic E' velocity corresponding to early diastolic relaxation and later diastolic A' velocity, corresponding to atrial contraction [84].

Tricuspid Valve Doppler

Tricuspid valve Doppler, an established clinical method of assessing diastolic filling, was included in the study as an adjunct to TDI. A tricuspid valve inflow pulse wave Doppler recording was obtained at the tip of the valve leaflets from an apical four-chamber view. Peak E and A wave velocities were then measured.

Echocardiographic determination of pulmonary artery pressure

Pulmonary artery pressure was estimated using two techniques; velocity of tricuspid regurgitation (TR) and pattern of ductal shunting. TR velocity was measured using continuous wave Doppler from an apical four chamber view. A modified Bernoulli equation was used to calculate the pressure gradient between the right atrium and

right ventricle [97]. Pulmonary artery pressure was quantified by adding 5 mmHg (an estimation of right atrial pressure) to this gradient. Pulmonary pressures were also qualitatively estimated from the pattern of shunting through the patent ductus arteriosus using pulse wave Doppler from a high left parasternal view [98]. Low velocity bi-directional shunting was considered to reflect systemic pulmonary artery pressures, whilst exclusive right-to-left shunting was considered to reflect suprasystemic pulmonary artery pressure.

Statistical analysis

Within each group myocardial velocities were summarised as mean and standard deviation (SD). Demographic data were compared between control and PHT groups using an unpaired t-test. RV function data was compared between control and PHT groups using Mann-Whitney tests.

RESULTS

Twenty-eight infants were recruited to the control group and 15 to the PHT group. There were no significant differences in gestation, corrected age in weeks, or weight between the two groups (Table 1).

Infants in the control group were older than the PHT group; this difference just reached statistical significance (P=0.05). There was a preponderance of female infants in the control group. Heart rate was not significantly different between control and PHT groups. Importantly, infants in the PHT group were more likely to be mechanically ventilated (93%), receiving inotropes (47%), and/or inhaled nitric

oxide (73%), than infants in the control group. Inotropes being administered were dopamine alone (four infants), dobutamine alone (one infant), milrinone alone (one infant) and combined dopamine and dobutamine (one infant).

In 11 infants PHT was secondary to congenital diaphragmatic hernia (CDH), and in the remaining infants secondary to meconium aspiration (one infant), alveolar capillary dysplasia (one infant) and idiopathic (two infants). All infants had PHT secondary to increased pulmonary vascular resistance. The predominance of infants with CDH in our study reflects the high numbers of infants with this diagnosis treated by our institution. The mechanisms, chronicity and severity of PHT in CDH may differ from other causes of PHT in infants [11]. CDH infants were included in analysis of the entire PHT group, but were also considered separately in a subgroup analysis.

Echocardiograms were well tolerated. One infant in the PHT group had a pulmonary artery pressure greater than two-thirds systemic arterial pressure, but less than systemic arterial pressure. The remaining 14 infants (94%) had a pulmonary arterial pressure equal to or greater than systemic arterial pressure.

PWTDI and tricuspid valve Doppler data were obtained in all infants. However, in one infant in the PHT group, artifact from high frequency oscillatory ventilation prevented data analysis. This problem was not encountered in other infants receiving high frequency oscillation.

Systolic myocardial velocities

Systolic PWTDI velocities in the RV and septum are summarized for the PHT and control groups in Table 2. IVV was significantly reduced in the RV in infants in the PHT group compared to controls. S wave velocity in the RV was also significantly reduced in the PHT group. No significant difference was observed in systolic PWTDI velocities in the interventricular septum. Subgroup analysis of the CDH infants alone, also demonstrated significant reduction in IVV and S velocities in the RV, compared to the control group.

Diastolic tricuspid valve flow and myocardial velocities

Tricuspid valve Doppler analysis in the PHT group demonstrated significant reduction in E wave velocity compared to controls, whilst A wave velocity was unchanged (Table 2). These findings indicate reduced early RV diastolic filling in the PHT group.

Analysis of diastolic TDI velocities revealed a reduction, or complete absence, of the E' velocity in the PHT group. In seven infants in the PHT group an E' wave was identifiable, and of significantly reduced velocity compared to the control group, in both the RV and interventricular septum. In the remaining eight PHT infants, E' waves were entirely absent (Figure 2). Absence of the E' wave was observed even in infants with lower heart rates, and therefore was considered to represent genuine absence of an early diastolic E'velocity, rather than E-A wave fusion. A' wave velocities were unchanged in TDI analysis. Subgroup analysis of CDH infants alone also demonstrated significant reductions in E' velocity compared to controls (Table 2).

In five infants in the PHT group prominent "post-systolic" positive velocities were present. These were positive myocardial velocities occurring after the systolic S wave, but before the E' or A' wave (Figure 2).

DISCUSSION

This is the first study to use TDI to assess RV function in sick newborn infants and has demonstrated significant reductions in systolic and early diastolic myocardial velocities in infants with pulmonary hypertension.

RV function, or dysfunction, is a principle determinant of illness severity in infants with PHT [34]. However, RV function assessment is rarely performed in infants with PHT, or is limited to qualitative assessment using 2-dimensional echocardiography. Alternative attempts to quantify RV function using ejection fraction and fractional shortening are time consuming and of questionable accuracy due to the RV's complex geometry [57].

TDI is a relatively new technique allowing direct measurement of myocardial velocities as measures of myocardial function [77]. TDI velocities correlate well with gold standard conductance catheter measures of ventricular function and are non-invasive and practical for use in the clinical setting [80, 81, 84]. Importantly, unlike other global measures of ventricular function, TDI measures allow separate analysis of systolic and diastolic function.

We have confirmed the feasibility of PWTDI in the RV in normal and PHT infants. Our measurements of systolic S velocity and diastolic E' and A' velocities in normal infants are in good agreement with previous normative infant data reported by Mori et al [94]. We were additionally able to measure IVV, as another measure of systolic function.

In the PHT group we observed reduced systolic myocardial velocities (IVV and S) and a significant reduction, or absence, of early diastolic (E') velocities. These changes may be considered to indicate inherent impairment of systolic and early diastolic function in the PHT group. However, the observed changes in myocardial TDI velocities might also be due to the direct effects of altered load, independent of changes in myocardial function. The degree of load-dependence of TDI velocities remains unclear – some animal studies have demonstrated relative load-independence, whilst others have demonstrated load-dependency at extremes of loading conditions [49, 82]. In infants, validation of TDI velocities against load-independent conductance catheter measures of RV function has not been performed, in this or prior studies, due to the unacceptably invasive nature of catheter techniques.

If the observed changes in TDI myocardial velocities genuinely reflect impaired systolic and diastolic function (independent of changes in loading) then this is consistent with prior demonstrations of systolic and diastolic RV dysfunction in animal models of PHT and adults with PHT [91-93]. One explanation for impaired early diastolic relaxation in the current study may be abnormally prolonged

contraction of the RV after pulmonary valve closure, producing the "post-systolic" positive myocardial velocities, which were observed in five infants in the PHT group.

It is of note that there was overlap of the ranges of myocardial velocities between PHT and control groups. This overlap between groups likely reflects the preservation of normal myocardial velocities in some PHT infants, demonstrating the variable nature of RV function in PHT [35, 145]. This finding emphasises the importance of directly assessing RV function, and not measuring pulmonary artery pressure alone, in infants with PHT.

It is important to note that the changes in myocardial velocities in the PHT group may not solely be due to increased afterload i.e. pulmonary artery pressure. The PHT infants were variably receiving a number of therapies (inotropes, pulmonary vasodilators and mechanical ventilation), which may have altered myocardial velocities either by directly altering myocardial function, or indirectly by modulating afterload. Specifically, dopamine, dobutamine and milrinone may, theoretically, affect systolic and diastolic myocardial function, whilst inhaled nitric oxide may have reduced afterload. It was beyond the scope of the current work to investigate the individual effects of these therapies on myocardial function though this remains an important area for future study, and potential use of TDI. Of note, the same findings of impaired RV systolic and diastolic myocardial velocities were present in

one infant in the PHT group who was not mechanically ventilated and was receiving no cardiac medications.

The findings of this study are relevant to the clinical management of infants with PHT, including those with CDH. Specifically, pulse wave TDI may have a practical role in the initial identification of RV dysfunction in infants with PHT, and in the assessment of response to therapies both in the clinical and research setting. For example, the use of pulmonary vasodilators and PGE1 (to maintain ductal patency) to reduce RV afterload [16, 114] has been proposed in infants with PHT and CDH. However, there have been no objective assessments of the direct effects of these therapies on RV function. TDI may represent a useful tool for measuring RV function in response to such new therapies.

The finding of diastolic and systolic abnormalities in PHT also has potential implications for the choice of drugs to directly improve RV function in PHT. Theoretically, agents which improve both inotropic and lusitropic (diastolic) function may be most effective. The phosphodiesterase inhibitor milrinone and calcium sensitizing agent levosimendan, are two such drugs [100, 147, 148]. Further studies are required to investigate the effects of these agents on RV function in infants with PHT.

Limitations of this study

Firstly, considering inter-group differences; the control group was older in postnatal age (but not gestational age or corrected gestational age) than the PHT group. This

age difference may have contributed to observed differences in RV velocities, as RV function is known to change in the first weeks of life [67]. It is also unclear what contribution, if any, the female preponderance in the control group may have made to the observed results. Additionally, the PHT group was a heterogenous group of infants with a variety of diagnoses. Only CDH infants were present in large enough numbers to allow sub-group analysis of myocardial velocities, and it is not known whether the same findings would be replicated in all other causes of PHT.

Secondly, the absence of long term outcome data, due to the limitations of study size and duration, means that no investigation of the utility of TDI velocities for outcome prediction or risk stratification could be made. The investigation of links between changes in TDI indices and outcome in infants with PHT is an important are for future study.

Thirdly, no assessment of observer variability was made. Specifically, since all data collection and analysis was performed by a single observer no assessment of inter-observer variability could be made. Future investigation of both intra- and inter-observer variability of TDI techniques is important before these can be recommended for clinical use in infants.

CONCLUSION

We have confirmed the feasibility of PWTDI in newborn infants and demonstrated reduced systolic and diastolic RV myocardial velocities in pulmonary hypertension, including in CDH. RV myocardial velocities were variable in PHT, likely indicating

variable RV function. The load-dependency and observer variability of TDI measures in infants requires further investigation, as do the utility of therapies which target both inotropic and lusitropic function in infants with PHT and RV dysfunction.

Acknowledgements

We acknowledge the assistance of Professor Colin Morley, Professor Dan Penny and Mr Bill Reid in the preparation of this manuscript.

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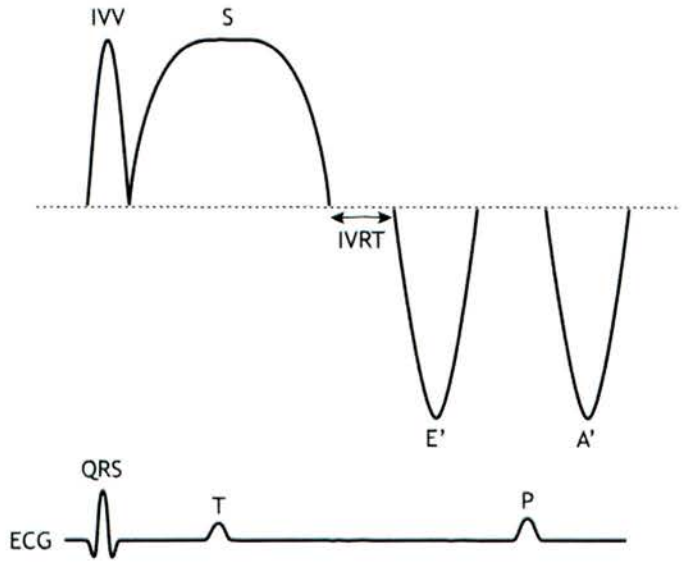
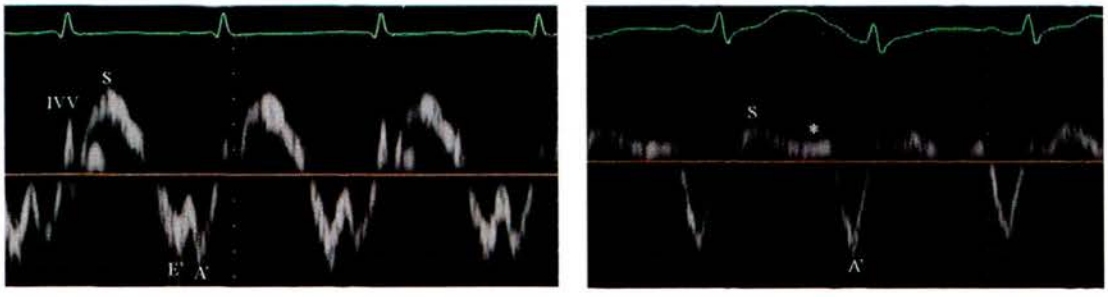
FIGURES

Figure 1: Schematic diagram of pulse wave TDI waveform and corresponding ECG.



IVV, isovolumic contraction velocity; S, systolic ejection velocity; E',early diastolic velocity; A', late diastolic velocity

Figure 2: PWTDI waveforms in normal and PHT infants.



Right image from normal infant; with E' and A' velocities clearly present.
Left image from infant with PHT, E' wave is absent and a post-systolic myocardial velocity (*) is present.

TABLES

Table 1: Demographic and therapeutic data

	Control Group n=28	PHT Group n=15	P
Gestation (weeks)	37.0 (3.5)	38.4 (2.0)	0.24
Postnatal age (days)	25 (20)	18 (28)	0.01
Corrected age (weeks)	40.5 (2.3)	40.9 (3.6)	0.80
Weight (kg)	3.22 (0.67)	3.34 (0.70)	0.70
Male/female	20/8	8/8	-
Heart rate (bpm)	140 (14)	148 (15)	0.16
Ventilated	**0 (0%)**	**14 (93%)**	-
Inotrope*	0 (0%)	7 (47%)	-
Inhaled nitric oxide	0 (0%)	11 (73%)	-

All demographic data mean (SD).

* Of those infants receiving inotropes: four infants on dopamine alone, one infant on dobutamine alone, one infant on milrinone alone, and one infant on dopamine and dobutamine.

Table 2: Echocardiographic measures of systolic and diastolic function

	Control Group n=28	PHT Group n=15	P	CDH subgroup n=11	P
Tricuspid Doppler velocities					
E wave velocity	0.52 (0.13)	0.14 (0.20)	<0.0001	0.13 (0.19)	<0.0001
A wave velocity	0.55 (0.11)	0.69 (0.22)	0.21	0.63 (0.18)	0.34
Systolic PWTDI data					
RV IVV	6.6 (1.1)	5.2 (1.3)	0.001	5.2 (1.5)	0.007
RV S	9.2 (1.9)	6.6 (1.3)	<0.0001	6.3 (1.3)	0.0001
Septal IVV	3.4 (0.5)	4.0 (1.1)	0.16	3.5 (0.7)	0.94
Septal S	5.0 (0.7)	5.1 (1.4)	0.82	5.0 (1.5)	0.46
Diastolic PWTDI data					
RV E' *	-8.5 (2.0)	-4.3 (2.3)	0.0001	-3.6 (1.8))	0.0006
RV A'	-10.1 (2.5)	-11.4 (4.2)	0.34	-10.8 (4.3)	0.68
Septal E' wave*	-5.4 (1.14)	-3.7 (0.5)	<0.0001	-3.6 (0.5)	0.0004
Septal A' wave	-6.3 (1.3)	-6.6 (2.9)	0.94	-6.4 (3.2)	0.561

All data mean (SD). All velocities are expressed as cm/sec.
* Data from those PHT infants in whom E' velocity could be identified. In remaining eight PHT infants no identifiable E' velocity present.

Appendix 3: Presentations of data at scientific meetings

- **Relationship between right ventricular function and pulmonary artery pressure in infants with pulmonary hypertension**

  Patel N, Mills J

  Perinatal Medicine (British Association of Perinatal Medicine / Neonatal Society), Harrogate, June 2008

- **Tissue Doppler imaging demonstrates systolic and diastolic dysfunction in infants with pulmonary hypertension**

  Patel N, Mills J, Cheung M

  Presented to the World Congress of Perinatal Medicine, Florence, September 2007

- **Assessment of right ventricular function in infants with pulmonary hypertension**

  Patel N, Mills J, Cheung M

  Presented to the Perinatal Society of Australia and New Zealand, April 2007

  Winner of Young Investigator Award